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SUBSTITUTE for PTO/SB/05 (4/98) Utility Patent Application Transmittal
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1 53(b))

Attorney Docket No	20413Y
First Inventor or Application Identifier	NEEPER, ET AL
Title	SYNTHETIC HUMAN PAPILLOMAVIRUS GENES
Express Mail Label No	EL523903475US

08/21/00

APPLICATION ELEMENTS
See MPEP chapter 600 concerning utility patent application contents

ADDRESS TO: **Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231**

1. [X] Fee Transmittal Form *(Submit an original, and a duplicate for fee processing)*
2. [X] Specification [*Total Pages* 30]
3. [X] Drawing(s) *(35 USC 113)* [*Total Sheets* 33]
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4. Oath or Declaration
   a. [X] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional with Box 14 completed)*
   [Note Box 5 below]
   i. [ ] DELETION OF INVENTOR(S)
   Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33 (b).
5. [ ] Incorporation By Reference *(useable if Box 4b is checked)*. The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference therein.
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7. [ ] Assignment papers (cover sheet & document(s))
8. [ ] 37 CFR 3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
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15. CORRESPONDENCE ADDRESS

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EXPRESS MAIL CERTIFICATE

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Washington, D.C. 20231

Deposit Acct. 13-2755
MERCK & CO., INC.
Our Case Docket No. 20413Y

Sir:

Transmitted herewith for filing under 37 C.F.R. §1.53(b) is the patent application of Inventor(s):
MICHAEL P. NEEPER, WILLIAM L. MCCLEMENTS, KATHRIN U. JANSEN, LOREN D. SCHULTZ, LING CHEN, XIN-MIN WANG

jc857 U.S. PTO 09/642405 08/21/00

For: SYNTHETIC HUMAN PAPILLOMAVIRUS GENES

For	Number Filed	Number Extra	Rate	Basic Fee $690
Total Claims	30 - 20 =	10 X	$18	= $180
Independent Claims	7 - 3 =	4 X	$78	= $312
Multiple Dependent Claims*		X	$260	=
* Add this fee if application contains any multiple dependent claims, regardless of number.		TOTAL FILING FEE ——→		$1,182

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☐ Under the provisions of 37 C.F.R. §1.53, this application is being filed without the declaration of each inventor.

Respectfully,

Joanne M. Giesser

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Date: August 21, 2000

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MAILED BY G. B. Crowley **DATE** 8/21/00

IN DUPLICATE

TITLE OF THE INVENTION

SYNTHETIC HUMAN PAPILLOMAVIRUS GENES

FIELD OF THE INVENTION

This invention relates to human papillomavirus (HPV) genes which have been codon-optimized for expression in a human cellular environment, and their use with adenoviral vectors and or plasmid vectors as vaccines.

BACKGROUND OF THE INVENTION

Papillomavirus infections occur in a variety of animals, including humans, sheep, dogs, cats, rabbits, snakes, monkeys and cows. Papillomaviruses infect epithelial cells, generally inducing benign epithelial or fibroepithelial tumors at the site of infection. Papillomaviruses are species specific infective agents; a human papillomavirus cannot infect a non-human.

Papillomaviruses are small (50-60nm), nonenveloped, icosahedral DNA viruses what encode up to eight early and two late genes. The open reading frames (ORFs) of the virus are designated E1 to E7 and L1 and L2, where "E" denotes early and "L" denotes late. L1 and L2 code for virus capsid proteins. The early genes are associated with functions such as viral replication and cellular transformation.

In humans, different HPV types cause distinct diseases, ranging from benign warts (for examples HPV types 1, 2, 3) to highly invasive genital and anal carcinomas (HPV types 16 and 18). At present there is not a satisfactory therapeutic regimen for these diseases.

Immunological studies in animals have shown that the production of neutralizing antibodies to papillomavirus antigens prevents infection with the homologous virus. However, development of a vaccine has been hindered by the difficulties associated with culture of the papillomavirus *in vitro*.

Vaccination is an effective form of disease prevention and has proven successful against several types of viral infection. However, to date, attempts to generate an effective HPV vaccine have not been entirely successful.

SUMMARY OF THE INVENTION

This invention relates to oligonucleotides which encode a human papillomavirus (HPV) protein which has been codon-optimized for efficient expression in a host cell; preferably the oligonucleotides are DNA. In one embodiment, the polynucleotides encode a protein which retains its wild-type amino acid sequence. In an alternate embodiment, the polynucleotides encode a mutated form of a HPV protein which has reduced protein function as compared to wild-type protein, but which maintains immunogenicity. This invention also relates to the mutated HPV proteins so encoded.

In preferred embodiments, the protein is selected from the group consisting of: L1, L2, E1, E2, E4, E5, E6 and E7 proteins. Particularly preferred are L1, L2, E2, and E7 proteins.

Another aspect of this invention is a vector carrying the polynucleotides encoding a codon-optimized HPV protein. Yet another aspect of this invention are host cells containing these vectors.

In a preferred embodiment, the vector is an adenoviral vector. In a particularly preferred embodiment, the adenoviral vector is a vaccine vector comprising an adenoviral genome with a deletion in the E1 region, and an insert in the E1 region, wherein the insert comprises an expression cassette comprising:

a) a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins or mutant forms thereof, wherein the polynucleotide is codon-optimized for expression in a human host cell; and

b) a promoter operably linked to the polynucleotide.

Another type of vector which is envisioned by this invention is a shuttle plasmid vector comprising a plasmid portion and an adenoviral portion, the adenoviral portion comprising: an adenoviral genome with a deletion in the E1 region, and an insert in the E1 region, wherein the insert comprises an expression cassette comprising:

a) a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins, wherein the polynucleotide is codon-optimized for expression in a human host cell; and

b) a promoter operably linked to the polynucleotide.

This invention also is directed to plasmid vaccine vectors, which comprise a plasmid portion and an expressible cassette comprising

a) a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins, wherein the polynucleotide is codon-optimized for expression in a human host cell; and

b) a promoter operably linked to the polynucleotide.

This invention also relates to vaccine compositions comprising a vector which carries the oligonucleotides to a human host, and allows for expression of the encoded protein. The protein is expressed in an amount sufficient to induce an immune response. In preferred embodiments, the vector is a plasmid vector or an adenoviral vector.

This invention also relates to a method of making a HPV protein comprising expressing in a host cell a synthetic polynucleotide encoding a human papillomavirus (HPV) protein, or mutated form of a HPV protein which has reduced protein function as compared to wild-type protein, but which maintains immunogenicity, the polynucleotide sequence comprising codons optimized for expression in a human host.

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BRIEF DECRIPTION OF THE DRAWINGS

FIGURE 1 is the nucleotide sequence of a codon-optimized HPV16 L1 gene (SEQ.ID.NO:1).

FIGURE 2 is the nucleotide sequence of a codon-optimized HPV16 E1 gene (SEQ.ID.NO:2). In this particular sequence, there are further mutations which changes the amino acid sequence of the expressed protein-- the glycine residue at position 428 has been converted to aspartic acid, and the tryptophan residue at position 439 is now arginine.

FIGURE 3 is the nucleotide sequence of a codon-optimized HPV16 E2 gene (SEQ.ID.NO:3). In this particular sequence, the glutamic acid residue at position 39 has been changed to an alanine, and the isoleucine residue at position 73 has also been changed to an alanine.

FIGURE 4 is the nucleotide sequence of a codon-optimized HPV16 E7 gene.(SEQ.ID.NO:4). In this particular sequence, the cysteine residue at position 24 has been changed to glycine, and the glutamic acid residue at position 26 has been changed to a glycine.

FIGURE 5 is the nucleotide sequence of a codon-optimized HPV6a E7 gene (SEQ.ID.NO:5).

FIGURE 6 is the nucleotide sequence of a codon-optimized HPV18 E7 gene (SEQ.ID.NO:6).

FIGURE 7 is the nucleotide sequence of a codon-optimized HPV6a E2 gene (SEQ.ID.NO:7).

FIGURE 8 is the nucleotide sequence of a codon-optimized HPV18 E2 gene (SEQ.ID.NO:8).

FIGURE 9 shows the results of immunoblot analysis of lysates of 293 cells transiently-transfected with native (lanes d, e, f) or synthetic (a, b, c) HPV16 L1 sequences in the expression vector V1Jns.

FIGURE 10 shows the results of immunoblot analysis of lysates of 293 cells transiently-transfected with native or synthetic HPV16 E1 sequences in the expression vector V1Jns. Lanes a and d contain native HPV16 E1 sequences; lanes b and e contain synthetic HPV16 E1, and lane c is a mock-transfected control.

FIGURE 11 shows the results of immunoblot analysis of lysates of 293 cells transiently-transfected with native or synthetic HPV16 E2 sequences in the expression vector V1Jns. Lane a is mock-infected; lane b is lacZ control; lane c contains a synthetic HPV16 E2 isolate #6; lane d contains synthetic HPV16 E2 isolate #11, and lane e has native HPV16 E2.

FIGURE 12 shows the results of immunoblot analysis of lysates of 293 cells transiently-transfected with native or synthetic HPV16 E7 sequences in the expression vector V1Jns. Lane a is mock-infected; lane b is lacZ control; lane c contains synthetic HPV16 E7 isolate #2; lane d is synthetic HPV16 E7 isolate 4; and lane e is native HPV16 E7.

FIGURE 13 shows the results of immunoblot analysis of lysates of 293 cells transiently-transfected with synthetic HPV6a E7 sequences in the expression vector V1Jns. Lanes b and c contain synthetic HPV6a E7 sequences; lane d contains a lacZ control, and lane a is a mock-transfected control.

FIGURE 14 shows the results of immunoblot analysis of lysates of 293 cells transiently-transfected with synthetic HPV18 E7 sequences in the expression vector V1Jns. Lanes b, c, d, and e contain synthetic HPV18 E7 sequences; lane f contains synthetic HPV16 E7 as an antibody control, and lane a is a mock-transfected control.

FIGURE 15 shows the results of immunoblot analysis of lysates of 293 cells transiently-transfected with synthetic HPV6a E2 sequences in the expression vector V1Jns. Lanes a and b contain synthetic E2 sequences; lane c is a beta-gal control, and lane d is mock-transfected.

FIGURE 16 shows the results of immunoblot analysis of lysates of 293 cells transiently-transfected with synthetic HPV18 E2 sequences in the expression vector V1Jns. Lane a is beta-gal control; lane b is mock transfected; and lanes c and d have synthetic sequences.

FIGURE 17 is a table of oligonucleotides (SEQ.ID.NOS:9-32) used to generate synthetic HPV16 L1.

FIGURE 18 is a table of oligonucleotides (SEQ.ID.NOS:33-64) used to generate synthetic HPV16 E1.

FIGURE 19 is a table of oligonucleotides (SEQ.ID.NOS:65-84) used to generate synthetic HPV16 E2.

FIGURE 20 is a table of oligonucleotides (SEQ.ID.NOS:85-90) used to generate synthetic HPV16 E7.

FIGURE 21 is a table of oligonucleotides (SEQ.ID.NOS:91-96) used to generate synthetic HPV6a E7.

FIGURE 22 is a table of oligonucleotides (SEQ.ID.NOS:97-102) used to generate synthetic HPV18 E7.

FIGURE 23 is a table of oligonucleotides (SEQ.ID.NOS:103-126) used to generate synthetic HPV6a E2.

FIGURE 24 is a table of oligonucleotides (SEQ.ID.NOS:127-150) used to generate synthetic HPV18 E2.

FIGURE 25 is a Western blot of JCL-031 cell lysate. Cell lysate was prepared from JCL-031 cells grown in selection medium containing 400μg/mL G418. The immunoblot was developed with anti-HPV 16 E2 (goat 248) antisera. Positions of molecular weight markers are indicated.

FIGURE 26 shows protection from JCL-031 cell-induced tumor outgrowth. E2 DNA- or control DNA-immunized mice were challenged by subcutaneous injection of 5 X10^5 JCL-031 cells into the left inguinal region. Beginning five days after this challenge, all animals were observed at two or three

day intervals until four weeks after inoculation. Tumors were detected and monitored by visual inspection, palpation of the inguinal region, and measurement of tumor diameter with calipers.

The term "promoter" as used herein refers to a recognition site on a DNA strand to which the RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences termed "enhancers" or inhibiting sequences termed "silencers".

The term "cassette" refers to the sequence of the present invention which contains the nucleic acid sequence which is to be expressed. The cassette is similar in concept to a cassette tape; each cassette has its own sequence. Thus by interchanging the cassette, the vector will express a different sequence. Because of the restrictions sites at the 5' and 3' ends, the cassette can be easily inserted, removed or replaced with another cassette.

The term "vector" refers to some means by which DNA fragments can be introduced into a host organism or host tissue. There are various types of vectors including plasmid, virus (including adenovirus), bacteriophages and cosmids.

The term "effective amount" means sufficient vaccine composition is introduced to produce the adequate levels of the polypeptide, so that an immune response results. One skilled in the art recognizes that this level may vary.

"Synthetic" means that the HPV gene has been modified so that it contains codons which are preferred for human expression. In many cases, the amino acids encoded by the gene remain the same. In some embodiments, the synthetic gene may encode a modified protein.

The term "native" means that the gene contains the DNA sequence as found in occurring in nature. It is a wild type sequence of viral origin.

DETAILED DESCRIPTION OF THE INVENTION

Synthetic DNA molecules encoding various HPV proteins are provided. The codons of the synthetic molecules are designed so as to use the codons preferred by the projected host cell, which is preferred embodiments is a human cell. The synthetic molecules may be used as a polynucleotide vaccine which provides effective immunoprophylaxis against papillomavirus infection through neutralizing

antibody and cell-mediated immunity. The synthetic molecules may be used as an immunogenic composition. This invention provides polynucleotides which, when directly introduced into a vertebrate *in vivo*, including mammals such as primates and humans, induce the expression of encoded proteins within the animal.

The gene encoding a L1, E1, E2 and/or E7 from any serotype HPV can be modified in accordance with this invention. It is preferred that the HPV chosen be one which is known to cause a pathological condition in humans. For this reason, it is preferred that the HPV gene be selected from the group consisting of: HPV6a, HPV6b, HPV11, HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV68 or variants thereof. The vaccine formulation of this invention may contain a mixture of HPV type protein genes (for example, genes from HPV6, 11, 16 and 18), and/or it may also contain a mixture of protein genes (i.e. L1, E1, E2, and/or E7).

Codon optimization

The wild-type sequences for many HPV genes are known. In accordance with this invention, HPV gene segments were converted to sequences having identical translated sequences but with alternative codon usage as defined by Lathe, 1985 "Synthetic Oligonucleotide Probes Deduced from Amino Acid Sequence Data: Theoretical and Practical Considerations" *J. Molec. Biol.* 183:1-12, which is hereby incorporated by reference. The methodology may be summarized as follows:

1. Identify placement of codons for proper open reading frame.
2. Compare wild type codon for observed frequency of use by human genes.
3. If codon is not the most commonly employed, replace it with an optimal codon for high expression in human cells.
4. Repeat this procedure until the entire gene segment has been replaced.
5. Inspect new gene sequence for undesired sequences generated by these codon replacements (e.g., "ATTTA" sequences, inadvertent creation of intron splice recognition sites, unwanted restriction enzyme sites, etc.) and substitute codons that eliminate these sequences.

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6. Assemble synthetic gene segments and test for improved expression.

In accordance with this invention, it has been found that the use of alternative codons encoding the same protein sequence may remove the constraints on expression of HPV proteins by human cells.

These methods were used to create the following synthetic gene segments for various papillomavirus genes creating a gene comprised entirely of codons optimized for high level expression. While the above procedure provides a summary of our methodology for designing codon-optimized genes for DNA vaccines, it is understood by one skilled in the art that similar vaccine efficacy or increased expression of genes may be achieved by minor variations in the procedure or by minor variations in the sequence.

In some embodiments of this invention, alterations have been made (particularly in the E-protein native protein sequences) to reduce or eliminate protein function while preserving immunogenicity. Mutations which decrease enzymatic function are known. Certain alterations were made for purposes of expanding safety margins and/or improving expression yield. These modifications are accomplished by a change in the codon selected to one that is more highly expressed in mammalian cells. In the case of HPV16 E1, for example two mutations were introduced: glycine at amino acid number 482 was changed to aspartic acid by conversion of GGC to GAC; and tryptophan was changed to arginine at position 439 by conversion of TGG to CGC.

For HPV16 E2, conversion of glutamic acid at position 39 to alanine and isoleucine at position 73 to alanine by conversion of both codons each to GCC.

For HPV16 E7, conversion of cysteine at position 24 to glycine and glutamic acid at position 26 to glycine was permitted by alteration of TGC and the GAG respectively both to GGC.

The codon-optimized genes are then assembled into an expression cassette which comprises sequences designed to provide for efficient expression of the protein in a human cell. The cassette preferably contains the codon-optimized gene, with related transcriptional and translations control sequences operatively linked to it, such as a promoter, and termination sequences. In a preferred embodiment, the promoter is the cytomegalovirus promoter with the intron A sequence (CMV-intA), although those skilled in the art will recognize that any of a

number of other known promoters such as the strong immunoglobulin, or other eukaryotic gene promoters may be used. A preferred transcriptional terminator is the bovine growth hormone terminator, although other known transcriptional terminators may also be used. The combination of CMVintA-BGH terminator is particularly preferred.

Examples of preferred gene sequences are given in SEQ.ID.NOS: 1-8.

VECTORS

In accordance with this invention, the expression cassette encoding at least one HPV protein is then inserted into a vector. The vector is preferably a plasmid or an adenoviral vector, although linear DNA linked to a promoter, or other vectors, such as adeno-associated virus or a modified vaccinia virus vector may also be used.

If the vector chosen is an adenovirus, it is preferred that the vector be a so-called first-generation adenoviral vector. These adenoviral vectors are characterized by having a non-functional E1 gene region, and preferably a deleted adenoviral E1 gene region. In some embodiments, the expression cassette is inserted in the position where the adenoviral E1 gene is normally located. In addition, these vectors optionally have a non-functional or deleted E3 region. The adenoviruses can be multiplied in known cell lines which express the viral E1 gene, such as 293 cells, or PerC.6 cells.

For convenience in manipulating the adenoviral vector, the adenovirus may be in a shuttle plasmid form. This invention is also directed to a shuttle plasmid vector which comprises a plasmid portion and an adenovirus portion, the adenovirus portion comprising an adenoviral genome which has a deleted E1 and optional E3 deletion, and has an inserted expression cassette comprising at least one codon-optimized HPV gene. In preferred embodiments, there is a restriction site flanking the adenoviral portion of the plasmid so that the adenoviral vector can easily be removed. The shuttle plasmid may be replicated in prokaryotic cells or eukaryotic cells.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the adenoviruses, shuttle plasmids and DNA immunogens of this invention.

If the vector chosen is plasmid DNA, it is preferred that the vector contain one or more promoters recognized by mammalian or insect cells. In a preferred embodiment, the plasmid would contain a strong promoter such as, but not limited to the CMV promoter. The gene to be expressed would be linked to such a promoter. An example of such a plasmid would be the mammalian expression plasmid V1Jns as described (J. Shiver et. al. 1996, in *DNA Vaccines*, eds., M. Liu, et al. N.Y. Acad. Sci., N.Y., 772:198-208 and is herein incorporated by reference).

In some embodiment of this invention, the both the vaccine plasmid and the adenoviral vectors may be administered to a vertebrate in order to induce an immune response. In this case, the two vectors are administered in a "prime and boost" regimen. For example the first type of vector is administered, then after a predetermined amount of time, for example, 1 month, 2 months, six months, or other appropriate interval, a second type of vector is administered. Preferably the vectors carry expression cassettes encoding the same polynucleotide or combination of polynucleotides.

Thus, another aspect of this invention is a method for inducing an immune response against human papillomavirus in a vertebrate, comprising

A) introducing into the vertebrate a first vector comprising a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins, wherein the polynucleotide is codon-optimized for expression in a human host cell;

B) allowing a predetermined amount of time to pass; and

C) introducing into the vertebrate a second vector comprising adenoviral vaccine vector comprising an adenoviral genome with a deletion in the E1 region, and an insert in the E1 region, wherein the insert comprises an expression cassette comprises

i) a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins or mutant forms thereof, wherein the polynucleotide is codon-optimized for expression in a human host cell; and

ii) a promoter operably linked to the polynucleotide.

In general, is preferred that the first vector be a plasmid vaccine vector and the second vector be an adenoviral vector. Thus this invention is directed to a method for inducing immune responses in a vertebrate comprising:

A) introducing into the vertebrate a plasmid vaccine, wherein the plasmid vaccine comprises a plasmid portion and an expression cassette portion, the expression cassette portion comprising:

i) a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins, wherein the polynucleotide is codon-optimized for expression in a human host cell; and

ii) a promoter operably linked to the polynucleotide;

B) allowing a predetermined amount of time to pass; and

C) introducing into the vertebrate an adenoviral vaccine vector comprising an adenoviral genome with a deletion in the E1 region, and an insert in the E1 region, wherein the insert comprises an expression cassette comprising:

i) a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins or mutant forms thereof, wherein the polynucleotide is codon-optimized for expression in a human host cell; and

ii) a promoter operably linked to the polynucleotide.

In yet another embodiment of the invention, the codon-optimized genes may be introduced into a recipient by way of a plasmid or adenoviral vector, as a "prime", and then a "boost" is accomplished by introducing into the recipient a polypeptide or protein which is essentially the same as that which is encoded by the codon-optimized gene. Fragments of a full length protein may be substituted, especially those which are immunogenic and/or include an epitope.

The amount of expressible DNA or transcribed RNA to be introduced into a vaccine recipient will depend partially on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of about 1 ng to 100 mg, and preferably about 10 μg to 300 μg of a plasmid vaccine vector is administered directly into muscle tissue. An effective dose for recombinant adenovirus is approximately 10^6-10^{12} particles and preferably about 10^7-10^{11} particles. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations may be provided. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration with adjuvants such as

interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine of this invention is also advantageous.

The vaccine vectors of this invention may be naked, that is, unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vaccine vectors to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline or the DNA may be associated with an adjuvant known in the art to boost immune responses, such as a protein or other carrier. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

The following examples are offered by way of illustration and are not intended to limit the invention in any manner.

EXAMPLES

EXAMPLE 1

Synthetic Gene Construction

Synthetic gene sequences for human papillomavirus proteins L1, E1, E2, and E7 were generated by reverse translation of amino acid sequences using the most frequently used codons found in highly expressed mammalian genes. (R. Lathe, 1985, *J. Mol. Biol.* 183:1-12, which is hereby incorporated by reference). Some adjustments to these codon-optimized sequences were made to introduce or remove restriction sites. Oligonucleotides based on these sequences were chemically synthesized (Midland Certified Reagents; Midland, TX) and assembled by PCR amplification. (J. Haas et. al., 1996, *Current Biology* 6:315-324; and *PCR Protocols*, M. Innis, et al, eds., Academic Press, 1990, both of which are hereby incorporated by reference).

Full-length sequences were cloned into the mammalian expression vector V1Jns (J. Shiver et. al. 1996, in *DNA Vaccines*, eds., M. Liu, et al. N.Y. Acad. Sci., N.Y., 772:198-208, which is hereby incorporated by reference) and sequenced by standard methodology. In cases where the actual sequence differed from the

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expected and resulted in amino acid substitution, that sequence was corrected by PCR mutagenesis as previously described (*PCR Protocols*, M. Innis, et al, eds., Academic Press, 1990, pg 177-180).

Protein expression was evaluated by transient transfection of equal quantities of plasmid DNA into 293 (transformed embryonic human kidney) cells which were harvested at 48 hr post DNA addition. Cell lysates were normalized to provide equal protein loadings. Analysis was by indirect immunofluorescence or immunoblot (Western) analysis using sera prepared to each of the HPV proteins. (Current Protocols in Molecular Biology, eds., F. Ausabel, *et. Al.*, John Wiley and Sons, 1998, which is hereby incorporated by reference).

EXAMPLE 2

Synthesis of HPV 16 L1

The gene encoding HPV16 L1 was prepared by the annealing and extension of the 14 oligomers listed in FIGURE 17. Five separate extension reactions were performed to create fragments of the gene, designated L1A, L1B, L1C, L1D and L1E by PCR using conditions similar to those described in EXAMPLE 3 and 4, below.

L1A was constructed using oligomer sequences MN4A1 (SEQ.ID.NO:9), MN4A2 (SEQ.ID.NO:16) and MN4A3 (SEQ.ID.NO:10) which were amplified using the oligomers MN604 (SEQ.ID.NO:32) and MN596 (SEQ.ID.NO:24).

L1B was constructed using oligomer sequences MN4A4 (SEQ.ID.NO:17), MN4A5 (SEQ.ID.NO:11) and MN4A6 (SEQ.ID.NO:18) and were amplified using the oligomers MN595 (SEQ.ID.NO:23) and MN598 (SEQ.ID.NO:26).

L1C was created using oligomer sequences MN4A7 (SEQ.ID.NO:12) and MN4A8 (SEQ.ID.NO:19) and were amplified using the oligomers MN597 (SEQ.ID.NO:25) and MN602 (SEQ.ID.NO:30).

L1D was created using oligomer sequences MN4A9 (SEQ.ID.NO:13), MN4A10 (SEQ.ID.NO:20) and MN4A11 (SEQ.ID.NO:14) which were amplified using the oligomers MN597 (SEQ.ID.NO:25) and MN602 (SEQ.ID.NO:30).

L1E was created using oligomer sequences MN4A12 (SEQ.ID.NO:21), MN4A13 (SEQ.ID.NO:15) and MN4A14 (SEQ.ID.NO:22) which were amplified using the oligomers MN601 (SEQ.ID.NO:29) and MN603 (SEQ.ID.NO:31).

Fragments L1A, L1B, L1C, L1D and L1E resulting from the PCR reactions were gel separated on low melting point agarose with the appropriately-sized products excised and purified using the Agarase™ method (Boehringer Mannheim Biochemicals) as recommended by the manufacturer. Fragments L1A, L1B and L1C were combined in a subsequent PCR reaction using oligomers MN604 (SEQ.ID.NO:32) and MN600 (SEQ.ID.NO:28) to assemble L1A-B-C; fragments L1D and L1E were assembled to L1D-E by subsequent PCR with the oligomers MN599 (SEQ.ID.NO:27) and MN603 (SEQ.ID.NO:31). The complete gene was then assembled by additional PCR reactions in which fragments L1A-B-C, L1D-E were combined with oligomers MN604 (SEQ.ID.NO:32) and MN603 (SEQ.ID.NO:31) in a final series of PCR reactions. The resulting 1.5 kb product was gel isolated, digested with Bgl II and subcloned into the V1Jns and sequenced. In instances where a mutation was observed, it was corrected by PCR mutagenesis as described in EXAMPLE 1. DNA was isolated from a clone with the correct HPV16 L1 DNA sequence and proper orientation within V1Jns for use in transient transfection assays as described in EXAMPLE 1.

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Transfection Results (HPV16 L1)

FIGURE 9 shows the HPV16 L1 immunoblot results of lysates of 293 cells transiently-transfected with the V1Jns plasmid containing either the native or the codon-optimized, synthetic HPV16 L1. Lanes a, b and c are the expression levels achieved using the synthetic HPV16 L1 expression construct. High levels of immunoreactive material are apparent in each of these lanes with the predominant band at approximately 55 kDa, consistent with the expected molecular weight for full-length HPV16 L1. In contrast, virtually no immunoreactive material is apparent in the lanes containing lysates transfected with the native HPV16 L1/ V1Jns plasmid (lanes d, e, and f). Since all cell lysate loadings were normalized and equivalent DNA amounts were used in the transfections, these findings indicate that the synthetic gene sequence greatly increased the levels of HPV16 L1 protein accumulation relative to that of the native gene sequence.

EXAMPLE 3

Synthesis of HPV 16 E1

The gene encoding the modified form of HPV16 E1 was assembled from a series of fragments: E1A, E1B, E1C, E1D, E1E and E1F, using the oligomers listed in FIGURE 18. E1A was formed by assembly of oligomers MN605 (SEQ.ID.NO:33), MN606 (SEQ.ID.NO:34) and MN607 (SEQ.ID.NO:35) and amplified using oligomers MN636 (SEQ.ID.NO:64) and MN624 (SEQ.ID.NO:52).

E1B was formed by assembly of oligomers MN608 (SEQ.ID.NO:36), MN609 (SEQ.ID.NO:37) and MN610 (SEQ.ID.NO:38) which were amplified with oligomers MN623 (SEQ.ID.NO:51) and MN626 (SEQ.ID.NO:54).

E1C was formed by assembly of oligomers MN611 (SEQ.ID.NO:39) and MN612 (SEQ.ID.NO:40) which were amplified with oligomers MN625 (SEQ.ID.NO:53) and MN628 (SEQ.ID.NO:56).

E1D was formed by assembly of oligomers MN613 (SEQ.ID.NO:41), MN614 (SEQ.ID.NO:42) and MN615 (SEQ.ID.NO:43) which were amplified with oligomers MN627 (SEQ.ID.NO:55) and MN630 (SEQ.ID.NO:58).

E1E was formed by assembly of oligomers MN616 (SEQ.ID.NO:44), MN617 (SEQ.ID.NO:45) and MN618 (SEQ.ID.NO:46) which were amplified with oligomers MN629 (SEQ.ID.NO:57) and MN632 (SEQ.ID.NO:60).

E1F was formed by assembly of oligomers MN619 (SEQ.ID.NO:47), MN620 (SEQ.ID.NO:48) and MN621(SEQ.ID.NO:49) which were amplified with oligomers MN631 (SEQ.ID.NO:59) and MN635 (SEQ.ID.NO:63).

Products of these PCR reactions were gel isolated and combined in subsequent rounds of PCR to form a 2 kb gene fragment encoding HPV16 E1 using methods described above. The resulting HPV16 E1 was inserted into the V1Jns expression vector as above and utilized in transient transfection studies as described in EXAMPLE 1.

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Transfection Results (HPV16 E1)

FIGURE 10 shows the HPV16 E1 immunoblot results of lysates of 293 cells transiently-transfected with the V1Jns plasmid containing either the native, or the codon-optimized, synthetic HPV16 E1. Lanes b and e are the expression levels achieved using the codon-optimized HPV16 E1 expression construct. High levels of HPV16 E1-specific immunostaining are apparent with a predominant band in lanes b and e at 72 kDa, consistent with the expected size for full-length HPV16 E1. In addition, there a number of smaller immunoreactive products which appear to be E1-specific as they are not observed in the mock transfected control (lane c).

A very different expression profile is observed in lysates of cells transfected with the native HPV16 E1/V1Jns construct, however. As shown in lanes a and d, only minimal amounts of immunoreactive material can be visualized which is not present in the mock transfection control. Since all cell lysate loadings were normalized and equivalent DNA amounts were used in the transfections these findings indicate that the synthetic gene sequence greatly increased the levels of HPV16 E1 protein accumulation relative to that of the native gene sequence.

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EXAMPLE 4

Synthesis of HPV 16 E2

Fragment AD. A 50 μl reaction containing oligonucleotides 13856-307-2A (SEQ.ID.NO:65), 13856-307-2B (SEQ.ID.NO:71), 13856-307-2C (SEQ.ID.NO:66), and 13856-307-2D (SEQ.ID.NO:72), at 150 nM each, dNTPs 0.5 mM each, Native buffer (Stratagene; La Jolla, CA) and 1 μL Native Pfu DNA polymerase (Stratagene) was incubated in a GeneAmp 9700 thermocycler (Perkin Elmer Applied Biosystems; Foster City, CA) under the following conditions: 95°C, 2 min.; 20 cycles of 95°C, 45 sec.; 55°C, 45 sec.; and 72°C, 2.5 min. Added to the reaction were primers 13856-307-2PA (SEQ.ID.NO:78) and 13856-307-2PD (SEQ.ID.NO:82) to a final concentration of 400 nM each, and 1 μL of Native Pfu DNA polymerase. The mixture was incubated for 2 min at 95°C and then 25 cycles of 95°C, 45 sec; 55°C, 45 sec; and 72°C, 2.5 min. The gel-isolated full-length fragment AD was amplified for 20 cycles under the same conditions using primers 13856-307-2PA (SEQ.ID.NO:78) and 13856-307-2PD (SEQ.ID.NO:82).

Fragment EH. A 50 μl reaction containing oligonucleotides 13856-307-2E (SEQ.ID.NO:67), 13856-307-2F (SEQ.ID.NO:73), 13856-307-2G (SEQ.ID.NO:68), and 13856-307-2H (SEQ.ID.NO:74) at 150 nM each, dNTPs 0.5 mM each, Native buffer and 1 μL Native Pfu DNA polymerase was incubated under the following conditions: 95°C, 2 min; 20 cycles of 95°C, 2 min.; 45 sec.; 55°C, 45 sec.; and 72°C, 2.5 min. Added to the reaction were primers 13856-307-2PE (SEQ.ID.NO:80) and 13856-307-2PH (SEQ.ID. NO:83) to a final concentration of 400 nM each, and 1 μL of Native Pfu DNA polymerase. The mixture was incubated for 95°C, 2 min.; then 25 cycles of 95°C, 45 sec.; 55°C, 45 sec.; and 72°C, 2.5 min.

Fragment IL. A 50 μl reaction containing oligonucleotides 13856-307-2I (SEQ.ID.NO:69), 13856-307-2J (SEQ.ID.NO:75), 13856-307-2K (SEQ.ID.NO:70), and 13856-307-2L (SEQ.ID.NO:76) at 150 nM each, dNTPs 0.5 mM each, Native buffer and 1 μL Native Pfu DNA polymerase was incubated under the following conditions: 95°C, 2 min; 20 cycles of 95°C, 2 min.; 45 sec.; 55°C, 45 sec.; and 72°C, 2.5 min. Added to the reaction were primers 13856-307-2PI (SEQ.ID.NO:81) and 13856-307-2PL (SEQ.ID.NO. 84) to a final concentration of 400 nM each, and 1 μL of Native Pfu DNA polymerase. The mixture was incubated at 95°C, 2 min.; then 25 cycles of 95°C, 45 sec.; 55°C, 45 sec.; and 72°C, 2.5 min.

Fragment AH. A 50 μl reaction containing 1.5μl each of AD and EH PCR products, dNTPs 0.5 mM each, Native buffer and 1 μL Native Pfu DNA polymerase was incubated under the following conditions: 95°C, 2 min; 20 cycles of 95°C, 45 sec.; 55°C, 45 sec.; and 72°C, 3.5 min. Added to the reaction were primers 13856-307-2PA (SEQ.ID.NO:78) and 13856-307-2PH (SEQ.ID.NO:83) to a final concentration of 400 nM each, and 1 μL of Native Pfu DNA polymerase. The mixture was incubated at 95°C, 2 min.; then 25 cycles of 95°C, 45 sec.; 55°C, 45 sec.; and 72°C, 3.5 min.

Fragment IM. A 50 μl reaction containing 1μl of IL PCR product, oligonucleotides 13856-307-2M (SEQ.ID.NO:77) and 13856-307-2PI (SEQ.ID.NO:81) each at a final concentration of 400 nM, dNTPs 0.5 mM each, Native buffer and 1 μL Native Pfu DNA polymerase was incubated under the following conditions: 95°C, 2 min; 25 cycles of 95°C, 45 sec.; 55°C, 45 sec.; and 72° C, 4 min.

Assembly of AM, full-length HPV16 E2. A 50 μl reaction containing 1.5μl each of fragments AH and IM, dNTPs 0.5 mM each, Native buffer and 1 μL

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Native Pfu DNA polymerase was incubated under the following conditions: 95°C, 2 min; 20 cycles of 95°C, 45 sec.; 55°C, 45 sec.; and 72°C, 4 min. Added to the reaction were primers 13856-307-2PA (SEQ.ID.NO:78) and 13856-307-2PM (SEQ.ID.NO:79) at a final concentration of 400 μM each, and 1 μL of Native Pfu DNA polymerase. The mixture was incubated at 95°C, 2 min.; then 25 cycles of 95° C, 45 sec.; 55°C, 45 sec.; and 72°C, 4 min. The resultant full-length fragment was isolated by electrophoresis through a 1.2% agarose gel the DNA recovered with a QIAquick column (Qiagen; Santa Clarita, CA) and subcloned into the expression vector V1Jns for evaluation.

Transfection Results (HPV16 E2)

FIGURE 11 shows the HPV16 E2 immunoblot results of lysates of 293 cells transiently-transfected with the V1Jns plasmid containing either the native, or the synthetic HPV16 E2. Lanes c and d are the expression levels achieved using the codon-optimized HPV16 E2 expression construct. High levels of HPV16 E2-specific immunostaining are visible which appear to be E2-specific as they are not observed in the mock transfected control (lane c).

A very different expression profile is observed in lysates of cells transfected with the native HPV16 E2/V1Jns construct, however. As shown in lane e, no immunoreactive material can be visualized. Since all cell lysate loadings were normalized and equivalent DNA amounts were used in the transfections, these findings indicate that the synthetic gene sequence greatly increased the levels of HPV16 E2 protein accumulation relative to those of the native gene sequence.

EXAMPLE 5

Synthesis of HPV 16 E7

The gene encoding HPV16 E7 was assembled from a series of fragments, made using oligomers listed in FIGURE 20.

A 50 μl reaction containing oligonucleotides 13856-307-7A (SEQ.ID.NO:85), 13856-307-7B (SEQ.ID.NO:87), 13856-307-7C (SEQ.ID.NO:86), and 13856-307-7D (SEQ.ID.NO:88) at 150 nM each, dNTPs 0.5 mM each, Native buffer (Stratagene; La Jolla, CA) and 1 μL Native Pfu DNA polymerase (Stratagene)

was incubated in a GeneAmp 9700 thermocycler (Perkin Elmer Applied Biosystems; Foster City, CA) under the following conditions: 95°C, 2 min; 20 cycles of 95°C, 45 sec.; 55°C, 45 sec. and 72°C, 2.5 min. Added to the reaction were primers 13856-307-7PA (SEQ.ID.NO:89) and 13856-307-7PD (SEQ.ID.NO:90) to a final concentration of 400 nM), and 1 μL of Native Pfu DNA polymerase. The mixture was incubated for 25 cycles of 95°C, 45 sec.; 55°C, 45 sec. and 72°C, 2.5 min.

The resultant full-length fragment was isolated by electrophoresis through a 1.2% agarose gel in TBE (Current Protocols in Molecular Biology, eds., F. Ausabel, *et. al.*, John Wiley and Sons, 1998, which is hereby incorporated by reference), stained with ethidium bromide, cut from the gel and recovered through a GenElute column (Supleco; Bellefonte, PA) and resuspended in 20 μl water. The sequence was further amplified in a 51 μl reaction containing 2 μl of fragment, 0.5μM each of oligonucleotides 13856-307-7PA (SEQ.ID.NO:89) and 13856-307-7PD, (SEQ.ID.NO:90) dNTPs 0.5mM each, Native buffer and Native Pfu DNA polymerase. The reaction was subjected to 20 cycles of 95°C, 45 sec.; 55°C, 45 sec. and 72°C, 2.5 min. The final amplified product isolated by electrophoresis as described above; the DNA recovered with a QIAquick column (Qiagen; Santa Clarita, CA) and subcloned into V1Jns.

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Transfection Results (HPV16 E7)

FIGURE 12 shows the HPV16 E7 immunoblot results of lysates of 293 cells transiently-transfected with the V1Jns plasmid containing either the native (lane e) or synthetic HPV16 E7 (lanes c and d). High levels of HPV16 E7-specific immunostaining are visible in the synthetic HPV16 E7 gene cell lysate lanes which are considerably more intense in appearance than that of the native HPV16 E7 gene cell lysate (lane e). Lanes a and b are negative transfection controls which show the antibody staining is specific to HPV16 E7 sequences. Since all cell lysate loadings were normalized and equivalent DNA amounts were used in the transfections, these findings indicate that the synthetic gene sequence greatly increased the levels of HPV16 E2 protein accumulation relative to those of the native gene sequence.

EXAMPLE 6

Synthesis of the E7 and E2 -encoding genes from HPV6a and HPV18:

The genes encoding HPV6a E7 and HPV 18 E7 were constructed using similar methods as described in EXAMPLE 4, except that the oligomers used to create the HPV6a E7 and HPV 18 E7 genes contain the sequences listed in FIGURE 21 and FIGURE 22, respectively. The construction of the synthetic genes encoding HPV6a E2 and HPV18 E2 was performed in a similar manner as detailed in EXAMPLE 5 using the oligomer sequences listed in FIGURE 23 and FIGURE 24 respectively.

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Transfection Results: HPV6a E7 and HPV 18 E7

FIGURE 13 shows the HPV6a E7 immunoblot results of lysates of 293 cells transiently-transfected with the V1Jns plasmid containing synthetic HPV6a E7 (lanes b and c). High levels of HPV6a E7-specific immunostaining are visible in the region expected for full-length HPV6a E7. A similar profile is found in FIGURE 14 by HPV18 E7 immunoblot analysis of lysates of 293 cells transiently-transfected with the V1Jns plasmid containing synthetic HPV6a E7 (lanes b, c, d and e). High levels of HPV18 E7-specific immunostaining are visible where full-length HPV18 E7 would be found as indicated by the location of the purified HPV18 protein control (lane f). There does not appear to be any stained material in the negative control lane a which indicates the staining in the other lanes is HPV18 E7-specific.

Expression of the synthetic gene encoding HPV6a E2 in V1Jns was evaluated by immunoblot analysis of transfected 293 cells which is shown in FIGURE 15. Lanes a and b are cell lysates of the synthetic HPV6a E2 transfectants; lanes c and d are negative controls. The analogous experiment is shown for HPV18 E2 expression in FIGURE 16. Lanes c and d are the cell lysates of transfections receiving the synthetic HPV18 E2 gene; lanes a and b are the negative controls. Both of these figures show measurable levels of E2 product accumulation when the codon-optimized, synthetic gene is expressed in mammalian cells.

These results indicate that the synthetic gene rebuilding is not limited to HPV16 genes. Rather, codon optimization of other HPV types also permits significant levels of E7 and E2 product accumulation in mammalian cells.

EXAMPLE 7

Construction of replication-defective FG-Ad expressing HPV antigen

Starting vectors

Shuttle vector pHCMVIBGHpA1 contains Ad5 sequences from bp1 to bp 341 and bp 3534 to bp 5798 with a expression cassette containing human cytomegalovirus (HCMV) promoter plus intron A and bovine growth hormone polyadenylation signal.

The adenoviral backbone vector pAdE1-E3- (also named as pHVad1) contains all Ad5 sequences except those nucleotides encompassing the E1 and E3 region.

Construction of Ad5. HPV16 E2

1. Construction of adenoviral shuttle plasmid pA1-CMVI-HPV16 E2 containing HPV16 E2 under the control of human CMV promoter and intron A.

The HPV16 E2 insert was excised from pV1JNS-HPV16 E2 by restriction enzyme Bgl II, EcoRI and then cloned into Bgl II, EcoRI digested shuttle vector pHCMVIBGHpA1.

2. Homologous recombination to generate plasmid form of recombinant adenoviral vector pAd-CMVI-HPV16 E2 containing HPV16 E2 expression cassette.

Shuttle plasmid pA1-CMVI- HPV16 E2 was digested with restriction enzymes BstZ17 and SgrA1 and then co-transformed into *E. coli* strain BJ5183 with linearized (ClaI digested) adenoviral backbone plasmid pAdE1-E3-. A colony was verified by PCR analysis. The vector was transformed to competent *E. coli* HB101 for large quantity production of the plasmid.

3. Generation of recombinant adenovirus Ad.CMVI- HPV16 E2 in 293 cells.

The pAd plasmid was linearized by restriction enzyme PacI and transfected to 293 cells using $CaPO_4$ method (Invitrogen kit). Ten days later, 10 plaques were picked and grown in 293 cells in 35-mm plates. PCR analysis of the adenoviral DNA showed virus were positive for HPV16 E2.

4. Evaluation of large scale recombinant adenovirus Ad.CMVI- HPV16 E2

A selected clone was grown into large quantities through multiple rounds of amplification in 293 cells. Expression of HPV16 E2 was also verified by ELISA and Western blot analysis of the 293 or COS cells infected with the recombinant adenovirus. The recombinant adenovirus was used for evaluation in mice and rhesus monkeys.

Method of Treatment

A person in need of therapeutic or prophylactic immunization against infection with human papillomavirus virus is injected with HPV DNA encoding all or part of; HPV L1, E1, E2, E4 or E7 and combinations thereof. The injection may be i.p., subcutaneous, intramuscular or intradermal. The HPV DNA may be used as a primer of the immune response or may be used as a booster of the immune response. The injection of DNA may antedate, coincide or follow injection of the person with a pharmaceutical composition comprising HPV virus like particles (containing only L1 protein or containing both L1 and L2 proteins, or containing mutant forms of one or more proteins), capsomeres, inactivated HPV, attenuated HPV, compositions comprising HPV-derived proteins, or combinations thereof.

EXAMPLE 8

The use of a synthetically-expressed HPV E protein as a model tumor antigen.

Generation of a tumor cell line that expresses HPV 16 E2.

A Not I-Hind III restriction digest fragment containing the synthetic coding sequence for HPV 16 E2 (see above) was ligated with Not I, Hind III digested expression vector pBJ/neo/CCR2B which has a neomycin resistance marker and drives the expression of the transgene with the HCMV immediate early promoter. The resultant plasmid, pBJ-16 E2, was characterized by restriction digestion, sequence analysis of the cloning junctions, and the ability to induce E2 protein expression in transiently-transfected A293 or CT26 cells. A stable cell line was generated transfection of CT26 cells using Lipofectamine (Gibco BRL). CT26 cells, a fully-transformed line derived from a BALB/c mouse colon carcinoma, have been widely used to present model tumor antigens. (Brattain et al., 1980 *Cancer Research*

40:2142-2146; Fearon, E. et al.,1988 *Cancer Research*, 48:2975-2980; both of which are incorporated by reference).

After 48 hours, cells were trypsinized, diluted 1:10, 1:100, 1:1000 or 1:10000 into medium and plated in 100mm^2 plates. After 24 hours, the medium was replaced with selection medium containing 400μg/mL G418. After two to three weeks, well-isolated colonies of cells were recovered using cloning rings and transferred to 48-well plates. One clone was positive for E2 expression by immunoblot analysis and was subjected to two further rounds of cloning by limiting dilution. One G418 resistant, E2-positive clonal isolate was used to established the cell line JCL-031. (FIGURE 25).

When inoculated into (syngeneic) BALB/c mice by subcutaneous injection, JCL-031 cell induced tumors with the kinetics similar to those as the parental CT26 line. Cells cultured from recovered tumors were G418 resistant and expressed E2.

Induction of immunity in mice by immunization with V1Jns:E2 DNA.

BALB/c mice were immunized multiple times by intramuscular injection with the DNA V1Jns:16E2 . Spleens from two randomly-chosen mice in each dose group were pooled, splenocytes prepared, and assayed in an murine interferon gamma Elispot assay. (Lalvani et al. 1997 *J. Exp. Med.* 186: 859-865; Forsthuber, T., et al 1996 *Science* 271: 1728-1730;. Chu, R. et al. 1997. *J. Exp. Med.* 186: 1623-1631, each of which is incorporated by reference.) Splenocyte cultures were incubated at 37°C for 24 hr. in the presence of a pool of 36 overlapping 20 amino acid residue peptides (final concentration, 4µg/mL each) scanning the full length of HPV 16 E2. Interferon gamma was captured on the substrate by monoclonal antibody (mAb) R4-6A2 (Pharmagin), and detected with biotinylated mAb XMG1.2 (Pharmagin) and a strepavidin-alkaline phosphatase conjugate (Pharmagin). Results are shown in Table A, below. The immunized mice developed $CD4^+$ immune responses to HPV (Table A, below).

Immunization with E2 DNA did not induce detectable anti-E2 antibody responses.

Table A

Dose Group	Immunization	E2-specific spots (per 10^6 cells)
1	E2 DNA 1	392
2	E2 DNA 2	96
3	E2 DNA 3	134
4	Control DNA 1	0
5	Control DNA 2	2

Protection from challenge with JCL-031 cells.

BALB/c mice, immunized with V1Jns:E2 DNA, or control DNA, were challenged by subcutaneous injection of 5 X10^5 JCL-031 cells into the left inguinal region. Tumor growth was monitored by palpation or caliper measurement for a four-week period. FIGURE 26 reports the fraction of each dose group that remained tumor free. The group that had been immunized with an E2-expressing plasmid was significantly protected from tumor development compared to the control group.

WHAT IS CLAIMED IS:

1. A synthetic polynucleotide comprising a sequence encoding a human papillomavirus (HPV) protein, or mutated form of a HPV protein which has reduced protein function as compared to wild-type protein, but which maintains immunogenicity, the polynucleotide sequence comprising codons optimized for expression in a human host.

2. A polynucleotide according to Claim 1 wherein the protein is selected from the group consisting of: L1, L2, E1, E2, E4, E5, E6 and E7.

3. A polynucleotide according to Claim 2 wherein the protein is selected from the group consisting of: L1, E1, E2, and E7.

4. A polynucleotide according to Claim 2 which is DNA.

5. A polynucleotide according to Claim 4 wherein the protein is L1 and is from an HPV selected from the group consisting of: HPV6a, HPV6b, HPV11, HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, and HPV68.

6. A polynucleotide according to Claim 5 wherein the protein is an HPV16 L1 protein.

7. A polynucleotide according to Claim 6 which comprises the polynucleotide of FIGURE 1 (SEQ.ID.NO: 1).

8. A polynucleotide according to Claim 4 wherein the protein is an E1 protein or a mutated E1 protein and is from an HPV selected from the group consisting of: HPV6a, HPV6b, HPV11, HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, and HPV 68.

9. A polynucleotide according to Claim 8 wherein the protein is a mutated form of E1.

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10. A polynucleotide according to Claim 8 which is an HPV16 E1 protein.

11. A polynucleotide according to Claim 10 which comprises the polynucleotide of FIGURE 2 (SEQ. ID.NO:2).

12. A polynucleotide according to Claim 4 wherein the protein is E2 protein or a mutated E2 protein and is from an HPV selected from the group consisting of: HPV6a, HPV6b, HPV11, HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, and HPV 68.

13. A polynucleotide according to Claim 12 wherein the protein is a mutated E2 protein.

14. A polynucleotide according to Claim 11 which is an HPV16 E2 mutated protein.

15. A polynucleotide according to Claim 14 which comprises the polynucleotide of FIGURE 3 (SEQ. ID.NO: 3).

16. A polynucleotide according to Claim 4 wherein the protein is E7 or an E7 mutant and is from an HPV selected from the group consisting of: HPV6a, HPV6b, HPV11, HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV68.

17. A polynucleotide according to Claim 16 wherein the protein is an HPV6a protein.

18. A polynucleotide according to Claim 17 which comprises the polynucleotide of FIGURE 4 (SEQ. ID.NO:4).

19. An adenoviral vaccine vector comprising an adenoviral genome with a deletion in the E1 region, and an insert in the E1 region, wherein the insert comprises an expression cassette comprising:

A) a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins or mutant forms thereof, wherein the polynucleotide is codon-optimized for expression in a human host cell; and

B) a promoter operably linked to the polynucleotide.

20. A vector according to Claim 19 wherein the adenoviral genome also contains a deleted E3 region.

21. A shuttle plasmid vector comprising a plasmid portion and an adenoviral portion, the adenoviral portion comprising: an adenoviral genome with a deletion in the E1 region, and an insert in the E1 region, wherein the insert comprises an expression cassette comprising:

A) a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins, wherein the polynucleotide is codon-optimized for expression in a human host cell; and

B) a promoter operably linked to the polynucleotide.

22. A vaccine plasmid comprising a plasmid portion and an expression cassette portion, the expression cassette portion comprising:

A) a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins, wherein the polynucleotide is codon-optimized for expression in a human host cell; and

B) a promoter operably linked to the polynucleotide.

23. A plasmid according to Claim 22 wherein the plasmid portion is V1Js.

24. A method for inducing immune responses in a vertebrate which comprises introducing between 1 ng and 100 mg of the polynucleotide of Claim 1 into the tissue of the vertebrate.

25. A method for inducing immune responses in a vertebrate which comprises introducing between 10^{11}-10^{12} particles of an adenoviral vector carrying the polynucleotide of Claim 1 into the tissue of the vertebrate.

26. A method for inducing an immune response against human papillomavirus in a vertebrate, comprising

A) introducing into the vertebrate a first vector comprising a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins, wherein the polynucleotide is codon-optimized for expression in a human host cell;

B) allowing a predetermined amount of time to pass; and

C) introducing into the vertebrate a second vector comprising adenoviral vaccine vector comprising an adenoviral genome with a deletion in the E1 region, and an insert in the E1 region, wherein the insert comprises an expression cassette comprises

i) a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins or mutant forms thereof, wherein the polynucleotide is codon-optimized for expression in a human host cell; and

ii) a promoter operably linked to the polynucleotide.

27. A method according to Claim 26 wherein the vertebrate is human.

28. A method for inducing immune responses in a vertebrate comprising

A) introducing into the vertebrate a plasmid vaccine, wherein the plasmid vaccine comprises a plasmid portion and an expression cassette portion, the expression cassette portion comprising:

i) a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins, wherein the polynucleotide is codon-optimized for expression in a human host cell; and

ii) a promoter operably linked to the polynucleotide;

B) allowing a predetermined amount of time to pass; and

C) introducing into the vertebrate an adenoviral vaccine vector comprising an adenoviral genome with a deletion in the E1 region, and an insert in the E1 region, wherein the insert comprises an expression cassette comprising:

i) a polynucleotide encoding an HPV protein selected from the group consisting of L1, E1, E2, and E7 proteins or mutant forms thereof, wherein the polynucleotide is codon-optimized for expression in a human host cell; and

ii) a promoter operably linked to the polynucleotide.

29. A method according to Claim 28 wherein the vertebrate is human.

30. A method of making a HPV protein comprising expressing in a host cell a synthetic polynucleotide encoding a human papillomavirus (HPV) protein, or mutated form of a HPV protein which has reduced protein function as compared to wild-type protein, but which maintains immunogenicity, polynucleotide sequence comprising codons optimized for expression in a human host.

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TITLE OF THE INVENTION

SYNTHETIC HUMAN PAPILLOMAVIRUS GENES

ABSTRACT OF THE DISCLOSURE

Synthetic DNA molecules encoding papillomavirus proteins are provided. The codons of the synthetic molecules are codons preferred by the projected host cell. The synthetic molecules may be used as a polynucleotide vaccine which provides effective immunoprophylaxis against papillomavirus infection through stimulation of neutralizing antibody and cell-mediated immunity.

FIGURE 1

SEQ.ID.NO:1 Sequence of the Codon-Optimized HPV16 L1

ATGAGCCTGTGGCTGCCCAGCGAGGCCACCGTGTACCTGCCTCCCGTGCCCG
TGAGCAAGGTGGTGAGCACCGACGAGTACGTGGCCCGCACCAACATCTACTA
CCACGCCGGCACCAGCCGCCTGCTGGCCGTGGGCCACCCCTACTTCCCCATC
AAGAAGCCCAACAACAACAAGATCCTGGTGCCCAAGGTGAGCGGCCTGCAG
TACCGCGTGTTCCGCATCCACCTGCCCGACCCCAACAAGTTCGGCTTCCCCGA
CACAAGCTTCTACAACCCCGACACCCAGCGCCTGGTGTGGGCCTGCGTGGGC
GTGGAGGTGGGCCGCGGCCAGCCCCTGGGCGTGGGCATCAGCGGCCACCCCC
TGCTGAACAAGCTGGACGACACCGAGAACGCCAGCGCCTACGCCGCCAACGC
CGGCGTGGACAACCGCGAGTGCATCAGCATGGACTACAAGCAGACCCAGCTG
TGCCTGATCGGCTGCAAGCCTCCCATCGGCGAGCACTGGGGCAAGGGCAGCC
CCTGCACCAACGTGGCCGTGAACCCCGGCGACTGCCCTCCCCTGGAGCTGAT
CAACACCGTGATCCAGGACGGCGACATGGTGGACACCGGCTTCGGCGCCATG
GACTTCACCACCCTGCAGGCCAACAAGAGCGAGGTGCCCCTGGACATCTGCA
CCAGCATCTGCAAGTACCCCGACTACATCAAGATGGTGAGCGAGCCCTACGG
CGACAGCCTGTTCTTCTACCTGCGCCGCGAGCAGATGTTCGTGCGCCACCTGT
TCAACCGCGCCGGCGCCGTGGGCGAGAACGTGCCCGACGACCTGTACATCAA
GGGCAGCGGCAGCACCGCCAACCTGGCCAGCAGCAACTACTTCCCCACTCCC
AGCGGCAGCATGGTGACCAGCGACGCCCAAATCTTCAACAAGCCCTACTGGC
TGCAGCGCGCCCAGGGCCACAACAACGGCATCTGCTGGGGCAACCAGCTGTT
CGTGACCGTGGTGGACACCACCCGCAGCACCAACATGAGCCTGTGCGCCGCC
ATCAGCACCAGCGAGACCACCTACAAGAACACCAACTTCAAGGAGTACCTGC
GCCACGGCGAGGAGTACGACCTGCAGTTCATCTTCCAGCTGTGCAAGATCAC
CCTGACCGCCGACGTGATGACCTACATCCACAGCATGAACAGCACCATCCTG
GAGGACTGGAACTTCGGCCTGCAGCCCCCTCCCGGCGGTACCCTGGAGGACA
CCTACCGCTTCGTGACCAGCCAGGCCATCGCCTGCCAGAAGCACACCCCTCC
CGCTCCCAAGGAGGATCCCCTGAAGAAGTACACCTTCTGGGAGGTGAACCTG
AAGGAGAAGTTCAGCGCCGACCTGGACCAGTTCCCCCTGGGCCGCAAGTTCC
TGCTGCAGGCCGGCCTGAAGGCCAAGCCCAAGTTCACCCTGGGCAAGCGCAA
GGCCACCCCCACCACCAGCAGCACCAGCACCACCGCCAAGCGCAAGAAGCG
CAAGCTGTAA

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FIGURE 2

SEQ.ID.NO:2 Codon-Optimized HPV16 E1-G482D,W439R Mutant:

ATGGCCGACCCCGCCGGCACCAACGGCGAGGAGGGCACCGGCTGCAACGGC
TGGTTCTACGTGGAGGCCGTGGTGGAGAAGAAGACCGGCGACGCCATCAGCG
ACGACGAGAACGAGAACGACAGCGACACCGGCGAGGACCTGGTGGACTTCA
TCGTGAACGACAACGACTACCTGACCCAGGCCGAGACCGAGACCGCCCACGC
CCTGTTCACCGCCCAGGAGGCCAAGCAGCACCGCGACGCCGTGCAGGTGCTG
AAGCGCAAGTACCTGGGCAGCCCCCTGAGCGACATCAGCGGCTGCGTCGACA
ACAACATCAGCCCCCGCCTGAAGGCCATCTGCATCGAGAAGCAGAGCCGCGC
CGCCAAGCGCCGCCTGTTCGAGAGCGAGGACAGCGGCTACGGCAACACCGA
GGTGGAGACCCAGCAGATGCTGCAGGTGGAGGGCCGCCACGAGACCGAGAC
CCCCTGCAGCCAGTACAGCGGCGGCAGCGGCGGCGGCTGCAGCCAGTACAGC
AGCGGCAGCGGCGGCGAGGGCGTGAGCGAGCGCCACACCATCTGCCAGACC
CCTCTGACCAACATCCTGAACGTGCTGAAGACCAGCAACGCCAAGGCCGCCA
TGCTGGCCAAGTTCAAGGAGCTGTACGGCGTGAGCTTCAGCGAGCTGGTGCG
CCCCTTCAAGAGCAACAAGAGCACCTGCTGCGACTGGTGCATCGCCGCCTTC
GGCCTGACCCCCAGCATCGCCGACAGCATCAAGACCCTGCTGCAGCAGTACT
GCCTGTACCTGCACATCCAGAGCCTGGCCTGCAGCTGGGGCATGGTGGTGCT
GCTGCTGGTGCGCTACAAGTGCGGCAAGAACCGCGAGACCATCGAGAAGCTG
CTGAGCAAGCTGCTGTGCGTGAGCCCCATGTGCATGATGATCGAGCCTCCCA
AGCTTCGCAGCACCGCCGCCGCCCTGTACTGGTACAAGACCGGCATCAGCAA
CATCAGCGAGGTGTACGGCGACACCCCCGAGTGGATCCAGCGCCAGACCGTG
CTGCAGCACAGCTTCAACGACTGCACCTTCGAGCTGAGCCAGATGGTGCAGT
GGGCCTACGACAACGACATCGTGGACGACAGCGAGATCGCCTACAAGTACGC
CCAGCTGGCCGACACCAACAGCAACGCCAGCGCCTTCCTGAAGAGCAACAGC
CAGGCCAAGATCGTGAAGGACTGCGCCACCATGTGCCGCCACTACAAGCGCG
CCGAGAAGAAGCAGATGAGCATGAGCCAGTGGATCAAGTACCGCTGCGACC
GCGTGGACGACGGCGGCGACCGCAAGCAGATCGTGATGTTCCTGCGCTACCA
GGGCGTGGAATTCATGAGCTTCCTGACCGCCCTGAAGCGCTTCCTGCAGGGC
ATCCCCAAGAAGAACTGCATCCTGCTGTACGGCGCCGCCAACACCGACAAGA
GCCTGTTCGGCATGAGCCTGATGAAGTTCCTGCAGGGCAGCGTGATCTGCTTC
GTGAACAGCAAGAGCCACTTCTGGCTGCAGCCCCTGGCCGACGCCAAGATCG
GCATGCTGGACGACGCCACCGTGCCCTGCTGGAACTACATCGACGACAACCT
GCGCAACGCCCTGGACGGCAACCTGGTGAGCATGGACGTGAAGCACCGCCCC
CTGGTGCAGCTGAAGTGCCCTCCCCTGCTGATCACCAGCAACATCAACGCCG
GCACCGACAGCCGCTGGCCCTACCTGCACAACCGCCTGGTGGTGTTCACCTTC
CCCAACGAGTTCCCCTTCGACGAGAACGGTAACCCCGTGTACGAGCTGAACG
ACAAGAACTGGAAGAGCTTCTTCAGCCGCACCTGGAGCCGCCTGAGCCTGCA
CGAGGACGAGGACAAGGAGAACGACGGCGACAGCCTGCCCACCTTCAAGTG
CGTGAGCGGCCAGAACACCAACACCCTGTAA

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FIGURE 3

SEQ.ID.NO.:3 Sequence of the Codon-Optimized HPV16E2-E39A,I73A Mutant:

ATGGAGACCCTGTGCCAGCGCCTGAACGTGTGCCAGGACAAGATCCTGACCC
ACTACGAGAACGACAGCACCGACCTGCGCGACCACATCGACTACTGGAAGCA
CATGCGCCTGGCCTGCGCCATCTACTACAAGGCCCGCGAGATGGGCTTCAAG
CACATCAACCACCAGGTGGTGCCCACCCTGGCCGTGAGCAAGAACAAGGCCC
TGCAGGCCGCCGAGCTGCAGCTGACCCTGGAGACCATCTACAACAGCCAGTA
CAGCAACGAGAAGTGGACCCTGCAGGACGTGAGCCTGGAGGTGTACCTGACC
GCCCCCACCGGCTGCATCAAGAAGCACGGCTACACCGTGGAGGTGCAGTTCG
ACGGCGACATCTGCAACACCATGCACTACACCAACTGGACCCACATCTACAT
CTGCGAGGAGGCCAGCGTGACCGTGGTGGAGGGCCAGGTGGACTACTACGG
CCTGTACTACGTGCACGAGGGCATCCGCACCTACTTCGTGCAGTTCAAGGAC
GACGCCGAGAAGTACAGCAAGAACAAGGTGTGGGAGGTGCACGCCGGCGGC
CAGGTGATCCTGTGCCCCACCAGCGTGTTCAGCAGCAACGAGGTGAGCAGCC
CCGAGACCATCCGCCAGCACCTGGCCAACCACAGCGCCGCCACCCACACCAA
GGCCGTGGCCCTGGGCACCGAGGAGACCCAGACCACCATCCAGCGCCCCCGC
AGCGAGCCCGACACCGGCAACCCCTGCCACACCACCAAGCTGCTGCACCGCG
ACAGCGTGGACAGCGCCCCCATCCTGACCGCCTTCAACAGCAGCCACAAGGG
CCGCATCAACTGCAACAGCAACACCACCCCCATCGTGCACCTGAAGGGCGAC
GCCAACACCCTGAAGTGCCTGCGCTACCGCTTCAAGAAGCACTGCAAGCTGT
ACACCGCCGTGAGCAGCACCTGGCACTGGACCGGCCACAACGTGAAGCACA
AGAGCGCCATCGTGACCCTGACCTACGACAGCGAGTGGCAGCGCGACCAGTT
CCTGAGCCAGGTGAAGATCCCCAAGACCATCACCGTGAGCACCGGCTTCATG
AGCATCTAA

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FIGURE 4

SEQ.ID.NO.:4 Codon-Optimized HPV16E7-C24G,E26G Mutant:

ATGCACGGCGACACCCCCACCCTGCACGAGTACATGCTGGACCTGCAGCCCG
AGACCACCGACCTGTACGGCTACGGCCAGCTGAACGACAGCAGCGAGGAGG
AGGACGAGATCGACGGCCCCGCCGGCCAGGCCGAGCCCGACCGCGCCCACT
ACAACATCGTGACCTTCTGCTGCAAGTGCGACAGCACCCTGCGCCTGTGCGT
GCAGAGCACCCACGTGGACATCCGCACCCTGGAGGACCTGCTGATGGGCACC
CTGGGCATCGTGTGCCCCATCTGCAGCCAGAAGCCCTAA

0964240S.0621.00

FIGURE 5

SEQ.ID.NO.:5 Codon-Optimized HPV6a E7 Gene:

ATGCACGGCCGCCACGTGACCCTGAAGGACATCGTGCTGGACCTGCAGCCTC
CCGACCCCGTGGGCCTGCACTGCTACGAGCAGCTGGTGGACAGCAGCGAGGA
CGAGGTGGACGAGGTGGACGGCCAGGACAGCCAGCCCCTGAAGCAGCACTT
CCAGATCGTGACCTGCTGCTGCGGCTGCGACAGCAACGTGCGCCTGGTGGTG
CAGTGCACCGAGACCGACATCCGCGAGGTGCAGCAGCTCCTGCTGGGTACCC
TGAACATCGTGTGCCCCATCTGCGCTCCCAAGACCTAA

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FIGURE 6

SEQ.ID.NO.:6 Codon-Optimized HPV18 E7 Gene:

ATGCACGGCCCCAAGGCCACCCTGCAGGACATCGTGCTGCACCTGGAGCCCC
AGAACGAGATCCCCGTGGACCTGCTGTGCCACGAGCAGCTGAGCGACAGCGA
GGAGGAGAACGACGAGATCGACGGCGTGAACCACCAGCACCTGCCCGCTCG
CAGGGCCGAGCCCCAGCGCCACACCATGCTGTGCATGTGCTGCAAGTGCGAG
GCCCGCATCGAGCTGGTGGTGGAGAGCAGCGCTGACGACCTGCGCGCCTTCC
AGCAGCTGTTCCTGAACACCCTGAGCTTCGTGTGCCCCTGGTGCGCCAGCCAG
CAGTAA

FIGURE 7

SEQ.ID.NO.:7 Codon-Optimized HPV6a E2 Gene:

ATGGAGGCCATCGCCAAGCGCCTGGACGCCTGCCAGGAGCAGCTGCTGGAGC
TGTACGAGGAGAACAGCACCGACCTGCACAAGCACGTGCTGCACTGGAAGTG
CATGCGCCACGAGAGCGTGCTGCTGTACAAGGCCAAGCAGATGGGCCTGAGC
CACATCGGCATGCAGGTGGTGCCTCCTCTGAAGGTGAGCGAGGCCAAGGGCC
ACAACGCCATCGAGATGCAGATGCACCTCGAGAGCCTGCTGCGCACCGAGTA
CAGCATGGAGCCCTGGACCCTGCAGGAGACCAGCTACGAGATGTGGCAGACC
CCTCCCAAGCGCTGCTTCAAGAAGCGCGGCAAGACCGTGGAGGTGAAGTTCG
ACGGCTGCGCCAACAACACCATGGACTACGTGGTGTGGACCGACGTGTACGT
GCAGGACAACGACACCTGGGTGAAGGTGCACAGCATGGTGGACGCCAAGGG
CATCTACTACACCTGTGGCCAGTTCAAGACCTACTACGTGAACTTCGTGAAGG
AGGCCGAGAAGTACGGCAGCACCAAGCACTGGGAGGTGTGCTACGGCAGCA
CCGTGATCTGCAGCCCCGCTAGCGTGAGCAGCACCACCCAGGAGGTGAGCAT
CCCCGAGAGCACCACCTACACTCCCGCCCAGACCAGCACCCTGGTGAGCAGC
AGCACCAAGGAGGACGCCGTGCAGACCCCTCCTCGCAAGCGCGCCCGCGGC
GTGCAGCAGAGCCCCTGCAACGCCCTGTGCGTGGCCCACATCGGCCCCGTGG
ATAGCGGCAACCACAACCTGATCACCAACAACCACGACCAGCACCAGCGCC
GCAACAACAGCAACAGCAGCGCCACTCCCATCGTGCAGTTCCAGGGCGAGAG
CAACTGCCTGAAGTGCTTCCGCTACCGCCTGAACGATCGCCACCGCCACCTGT
TCGACCTGATCAGCAGCACCTGGCACTGGGCCAGCAGCAAGGCTCCCCACAA
GCACGCCATCGTGACCGTGACCTACGACAGCGAGGAGCAGCGCCAGCAGTTC
CTGGACGTGGTGAAGATCCCTCCCACCATCAGCCACAAGCTGGGCTTCATGA
GCCTGCACCTGCTGTAA

FIGURE 8

SEQ.ID.NO.:8 Codon-Optimized HPV18 E2 Gene:

ATGCAGACTCCCAAGGAGACCCTGAGCGAGCGCCTGAGCGCCCTGCAGGACA
AGATCATCGACCACTACGAGAACGACAGCAAGGACATCGACAGCCAGATCC
AGTACTGGCAGCTGATCCGCTGGGAGAACGCCATCTTCTTCGCCGCTCGCGA
GCACGGGATCCAGACCCTGAACCACCAGGTGGTGCCCGCCTACAACATCAGC
AAGAGCAAGGCCCACAAGGCCATCGAGCTGCAGATGGCCCTGCAGGGCCTG
GCCCAGAGCGCCTACAAGACCGAGGACTGGACCCTGCAGGACACCTGCGAG
GAGCTGTGGAACACCGAGCCCACCCACTGCTTCAAGAAGGGAGGCCAGACC
GTGCAGGTGTACTTCGACGGCAACAAGGACAACTGCATGAACTACGTGGCCT
GGGACAGCGTGTACTACATGACCGACGCCGGCACCTGGGACAAGACCGCCAC
CTGCGTGAGCCACCGCGGCCTGTACTACGTGAAGGAGGGCTACAACACCTTC
TACATCGAGTTCAAGAGCGAGTGCGAGAAGTACGGCAACACCGGCACCTGG
GAGGTGCACTTCGGCAACAACGTGATCGACTGCAACGACAGCATGTGCAGCA
CCAGCGACGACACCGTGAGCGCCACCCAGCTGGTGAAGCAGCTGCAGCACAC
TCCCAGCCCCTACAGCAGCACCGTGAGCGTGGGCACCGCCAAGACCTACGGC
CAGACCAGCGCCGCCACTCGCCCTGGCCACTGCGGCCTGGCCGAGAAGCAGC
ACTGCGGGCCCGTGAACCCTCTGCTGGGCGCCGCCACCGCCACCGGCAACAA
CAAGCGCCGCAAGCTGTGCAGCGGCAACACCACTCCCATCATCCACCTGAAG
GGCGACCGCAACAGCCTGAAGTGCCTGCGGTACCGCCTGCGCAAGCACAGCG
ACCACTACCGCGACATCAGCAGCACCTGGCACTGGACCGGCGCCGGGAACGA
GAAGACCGGCATCCTGACCGTGACCTACCACAGCGAGACCCAGCGCACCAAG
TTCCTGAACACCGTGGCCATCCCCGACAGCGTGCAGATCCTGGTGGGCTACA
TGACCATGTAA

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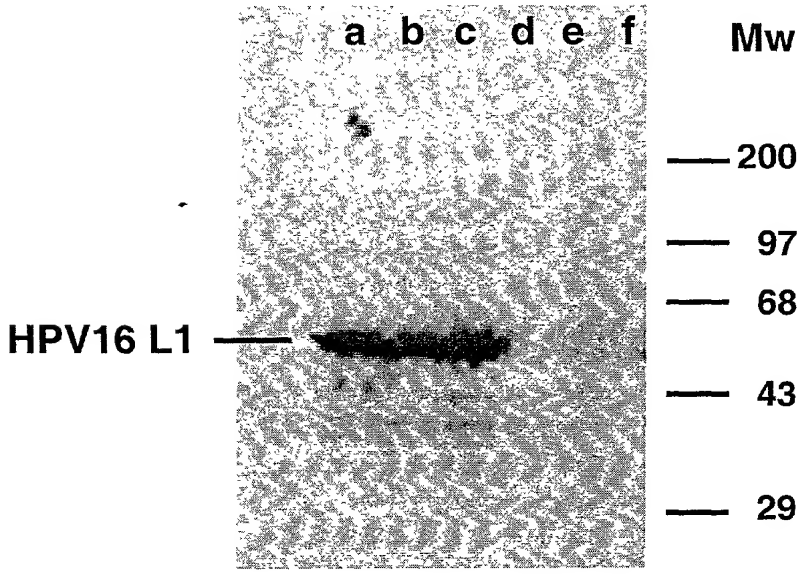
FIGURE 9.

Comparison of protein expression of native and synthetic HPV16 L1 genes



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Docket Alarm, LLC

FIGURE 10

Comparison of protein expression
of native and synthetic HPV 16 E1 genes

a b c d e

HPV 16 E1 —

— 188

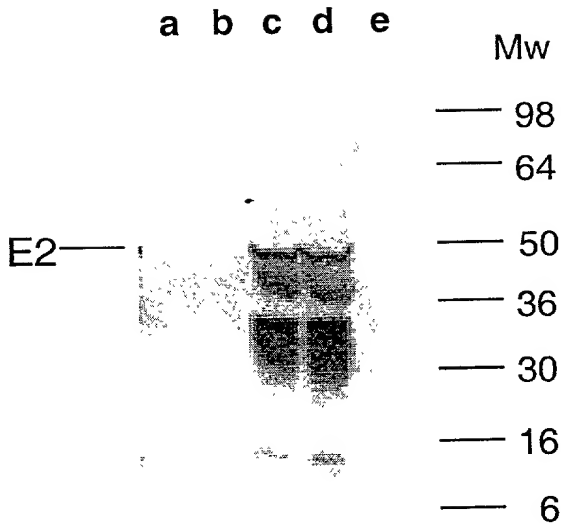
— 97

— 52

— 33

— 21

FIGURE 11.

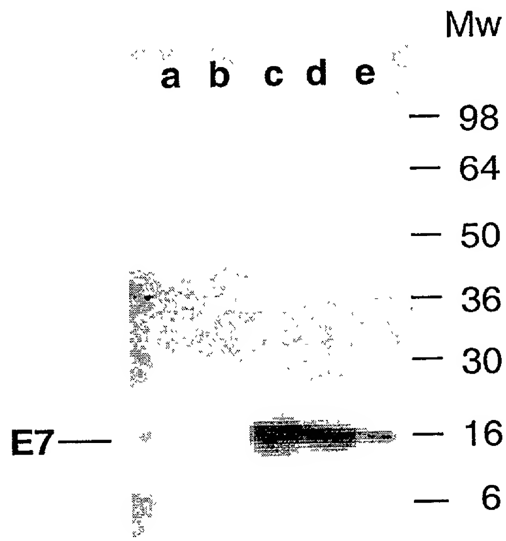


a. mock
b. lacZ
c. synthetic 16 E2 isolate 6
d. synthetic 16 E2 isolate 11
e native 16 E2

xmw98.75 16557-27

FIGURE 12

Comparison of protein expression of native and synthetic HPV16 E7 genes



a. mock
b. lacZ
c. synthetic HPV16 E7 isolate 2
d. synthetic HPV16 E7 isolate 4
e. native HPV16 E7

xmw98.75 16557-27

FIGURE 13

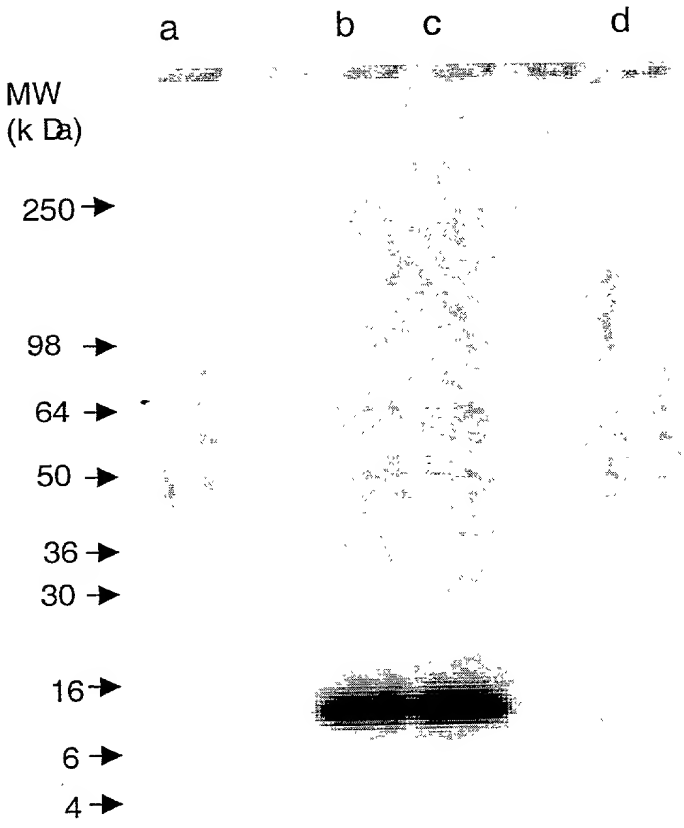
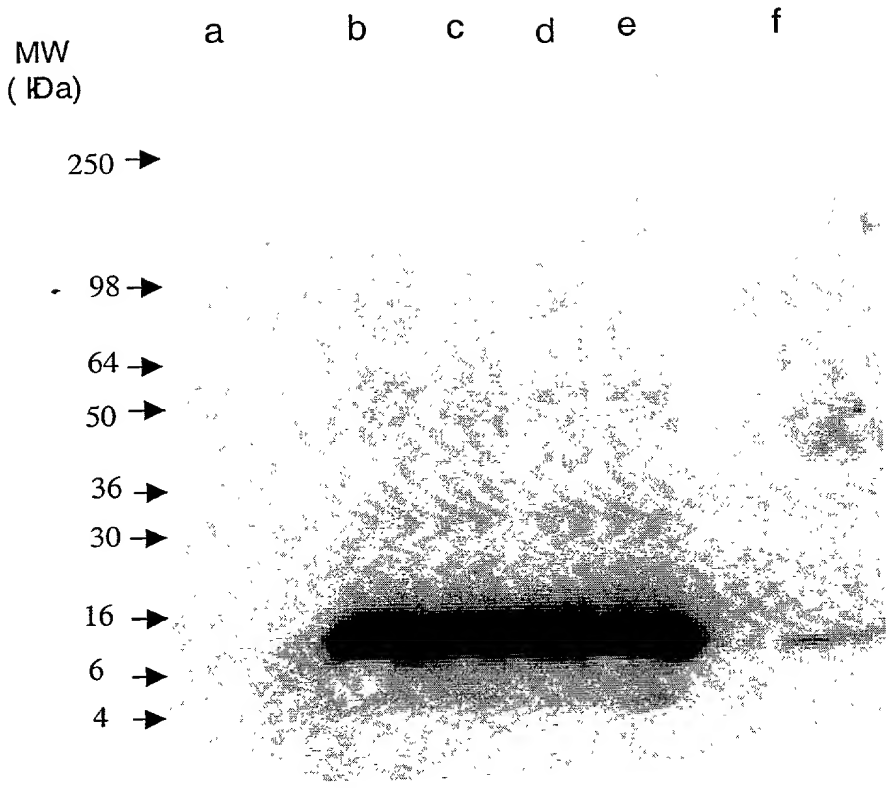


FIGURE 14



Expression of synthetic HPV 6 E2 gene

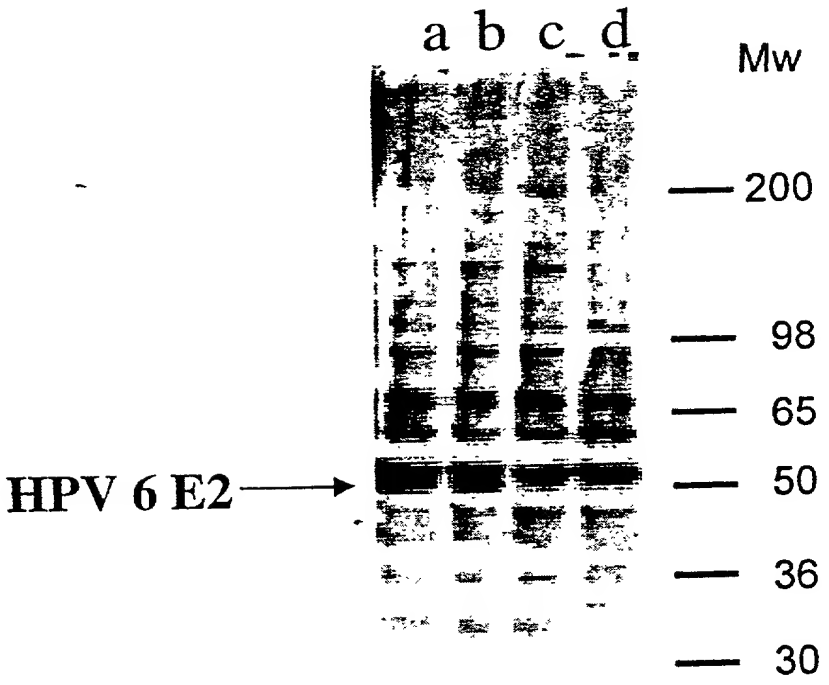


Figure 15. Expression of a synthetic gene encoding HPV 6 E2 protein. 293 cells were transfected with synthetic HPV 6 E2 or control plasmids or were mock transfected. Cell lysates were prepared 48 hr. later, fractionated by SDS PAGE and analyzed by immunoblotting using a goat anti- 6E2 antiserum as first antibody. a. 6 E2-5.4; b. 6E2-5.5; c. beta-gal; d. mock.

DO NOT SCAN

Expression of synthetic HPV 18 E2 gene

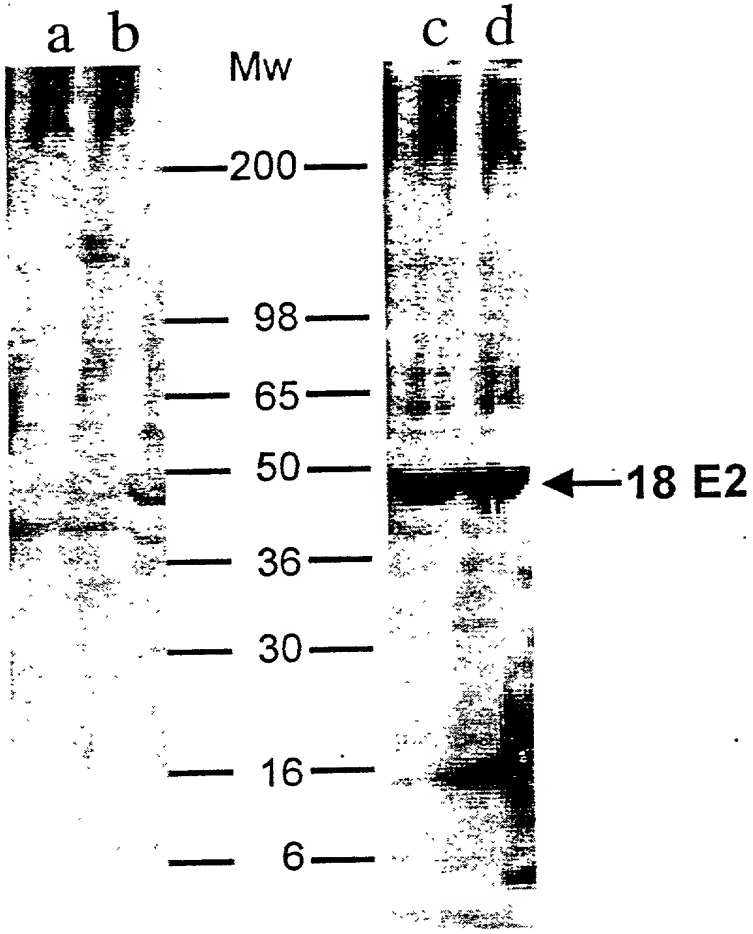


Figure 16. Expression of a synthetic gene encoding HPV 18 E2 protein. 293 cells were transfected with synthetic HPV 18 E2 or control plasmids or were mock transfected. Cell lysates were prepared 48 hr. later, fractionated by SDS PAGE and analyzed by immunoblotting using a goat anti- 18E2 antiserum as first antibody. a. beta-gal; b. mock; c. 18 E2-4.4; d. 18E2-4.5.

FIGURE 17

HPV16 L1 Gene-Building Oligomers

MN4A1 (SEQ.ID.NO:9) 5' ATG AGC CTG TGG CTG CCC AGC GAG GCC ACC GTG TAC CTG CCT CCC GTG CCC GTG AGC AAG GTG GTG AGC ACC GAC GAG TAC GTG GCC CGC ACC AAC ATC TAC TAC CAC GCC GGC ACC AGC CGC CTG CTG 3'

MN4A3 (SEQ.ID.NO:10) 5' CGC ATC CAC CTG CCC GAC CCC AAC AAG TTC GGC TTC CCC GAC ACA AGC TTC TAC AAC CCC GAC ACC CAG CGC CTG GTG TGG GCC TGC GTG GGC GTG GAG GTG GGC CGC GGC CAG CCC CTG GGC GTG GGC 3'

MN4A5 (SEQ.ID.NO:11) 5' GAG TGC ATC AGC ATG GAC TAC AAG CAG ACC CAG CTG TGC CTG ATC GGC TGC AAG CCT CCC ATC GGC GAG CAC TGG GGC AAG GGC AGC CCC TGC ACC AAC GTG GCC GTG AAC CCC GGC GAC TGC CCT CCC 3'

MN4A7 (SEQ.ID.NO:12) 5' GCC AAC AAG AGC GAG GTG CCC CTG GAC ATC TGC ACC AGC ATC TGC AAG TAC CCC GAC TAC ATC AAG ATG GTG AGC GAG CCC TAC GGC GAC AGC CTG TTC TTC TAC CTG CGC CGC GAG CAG ATG TTC GTG CGC 3'

MN4A9 (SEQ.ID.NO:13) 5' GCC AGC AGC AAC TAC TTC CCC ACT CCC AGC GGC AGC ATG GTG ACC AGC GAC GCC CAA ATC TTC AAC AAG CCC TAC TGG CTG CAG CGC GCC CAG GGC CAC AAC AAC GGC ATC TGC TGG GGC AAC CAG CTG 3'

MN4A11 (SEQ.ID.NO:14) 5' GAG TAC CTG CGC CAC GGC GAG GAG TAC GAC CTG CAG TTC ATC TTC CAG CTG TGC AAG ATC ACC CTG ACC GCC GAC GTG ATG ACC TAC ATC CAC AGC ATG AAC AGC ACC ATC CTG GAG GAC TGG AAC TTC GGC CTG 3'

MN4A13 (SEQ.ID.NO:15) 5' GCT CCC AAG GAG GAT CCC CTG AAG AAG TAC ACC TTC TGG GAG GTG AAC CTG AAG GAG AAG TTC AGC GCC GAC CTG GAC CAG TTC CCC CTG GGC CGC AAG TTC CTG CTG CAG GCC GGC CTG AAG GCC AAG CCC AAG 3'

MN4A2 (SEQ.ID.NO:16) 5' GTT GGG GTC GGG CAG GTG GAT GCG GAA CAC GCG GTA CTG CAG GCC GCT CAC CTT GGG CAC CAG GAT CTT GTT GTT GTT GGG CTT CTT GAT GGG GAA GTA GGG GTG GCC CAC GGC CAG CAG GCG GCT GGT GCC GGC 3'

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MN4A4 (SEQ.ID.NO:17) 5' CTT GTA GTC CAT GCT GAT GCA CTC GCG GTT GTC CAC GCC GGC GTT GGC GGC GTA GGC GCT GGC GTT CTC GGT GTC GTC CAG CTT GTT CAG CAG GGG GTG GCC GCT GAT GCC CAC GCC CAG GGG CTG GCC GCG 3'

MN4A6 (SEQ.ID.NO:18) 5' CAG GGG CAC CTC GCT CTT GTT GGC CTG CAG GGT GGT GAA GTC CAT GGC GCC GAA GCC GGT GTC CAC CAT GTC GCC GTC CTG GAT CAC GGT GTT GAT CAG CTC CAG GGG AGG GCA GTC GCC GGG GTT CAC 3'

MN4A8 (SEQ.ID.NO:19) 5' GGG AGT GGG GAA GTA GTT GCT GCT GGC CAG GTT GGC GGT GCT GCC GCT GCC CTT GAT GTA CAG GTC GTC GGG CAC GTT CTC GCC CAC GGC GCC GGC GCG GTT GAA CAG GTG GCG CAC GAA CAT CTG CTC GCG 3'

MN4A10 (SEQ.ID.NO:20) 5' CTC CTC GCC GTG GCG CAG GTA CTC CTT GAA GTT GGT GTT CTT GTA GGT GGT CTC GCT GGT GCT GAT GGC GGC GCA CAG GCT CAT GTT GGT GCT GCG GGT GGT GTC CAC CAC GGT CAC GAA CAG CTG GTT GCC CCA GCA GAT GCC 3'

MN4A12 (SEQ.ID.NO:21) 5' CTT CAG GGG ATC CTC CTT GGG AGC GGG AGG GGT GTG CTT CTG GCA GGC GAT GGC CTG GCT GGT CAC GAA GCG GTA GGT GTC CTC CAG GGT ACC GCC GGG AGG GGG CTG CAG GCC GAA GTT CCA GTC CTC CAG 3'

MN4A14 (SEQ.ID.NO:22) 5' CAC TAG AGA TCT GAA TTC TTA CAG CTT GCG CTT CTT GCG CTT GGC GGT GGT GCT GGT GCT GCT GGT GGT GGG GGT GGC CTT GCG CTT GCC CAG GGT GAA CTT GGG CTT GGC CTT CAG GCC GGC 3'

MN595 (SEQ.ID.NO:23) 5' CGC GGC CAG CCC CTG GGC GTG 3'

MN596 (SEQ.ID.NO:24) 5' GCC CAC GCC CAG GGG CTG GCC GCG 3'

MN597 (SEQ.ID.NO:25) 5' GCC AAC AAG AGC GAG GTG CCC 3'

MN598 (SEQ.ID.NO:26) 5' CAG GGG CAC CTC GCT CTT GTT GGC 3'

MN599 (SEQ.ID.NO:27) 5' GCC AGC AGC AAC TAC TTC CCC AC 3'

MN600 (SEQ.ID.NO:28) 5' GGG AGT GGG GAA GTA GTT GCT GC 3'

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FIG. 17, CTD. 3/3

MN601 (SEQ.ID.NO:29) 5' CTG GAG GAC TGG AAC TTC GGC CTG 3'

MN602 (SEQ.ID.NO:30) 5' CAG GCC GAA GTT CCA GTC CTC CAG 3'

MN603 (SEQ.ID.NO:31) 5' CAC TAG AGA TCT GAA TTC TTA CAG C 3'

MN6O4 (SEQ.ID.NO:32) 5' CAT CTC AGA TCT GCC ACC ATG AGC CTG TGG CTG CCC AG 3'

FIGURE 18

HPV16E1 Gene-building Oligomers

MN605 (SEQ.ID.NO:33) 5' ATG GCC GAC CCC GCC GGC ACC AAC GGC GAG GAG GGC ACC GGC TGC AAC GGC TGG TTC TAC GTG GAG GCC GTG GTG GAG AAG AAG ACC GGC GAC GCC ATC AGC GAC GAC GAG AAC GAG AAC GAC AGC GAC 3'

MN606 (SEQ.ID.NO:34) 5' GTG CTG CTT GGC CTC CTG GGC GGT GAA CAG GGC GTG GGC GGT CTC GGT CTC GGC CTG GGT CAG GTA GTC GTT GTC GTT CAC GAT GAA GTC CAC CAG GTC CTC GCC GGT GTC GCT GTC GTT CTC GTT CTC GTC 3'

MN607(SEQ.ID.NO:35) 5' GCC CAG GAG GCC AAG CAG CAC CGC GAC GCC GTG CAG GTG CTG AAG CGC AAG TAC CTG GGC AGC CCC CTG AGC GAC ATC AGC GGC TGC GTC GAC AAC AAC ATC AGC CCC CGC CTG AAG GCC ATC TGC ATC GAG 3'

MN608 (SEQ.ID.NO:36) 5' CTC GTG GCG GCC CTC CAC CTG CAG CAT CTG CTG GGT CTC CAC CTC GGT GTT GCC GTA GCC GCT GTC CTC GCT CTC GAA CAG GCG GCG CTT GGC GGC GCG GCT CTG CTT CTC GAT GCA GAT GGC CTT CAG GC 3'

MN609 (SEQ.ID.NO:37) 5' CAG GTG GAG GGC CGC CAC GAG ACC GAG ACC CCC TGC AGC CAG TAC AGC GGC GGC AGC GGC GGC GGC TGC AGC CAG TAC AGC AGC GGC AGC GGC GGC GAG GGC GTG AGC GAG CGC CAC ACC ATC TGC CAG ACC 3'

MN610 (SEQ.ID.NO:38): 5' CTT GAA GGG GCG CAC CAG CTC GCT GAA GCT CAC GCC GTA CAG CTC CTT GAA CTT GGC CAG CAT GGC GGC CTT GGC GTT GCT GGT CTT CAG CAC GTT CAG GAT GTT GGT CAG AGG GGT CTG GCA GAT GGT GTG GCG 3'

MN611 (SEQ.ID.NO:39) 5' GAG CTG GTG CGC CCC TTC AAG AGC AAC AAG AGC ACC TGC TGC GAC TGG TGC ATC GCC GCC TTC GGC CTG ACC CCC AGC ATC GCC GAC AGC ATC AAG ACC CTG CTG CAG CAG TAC TGC CTG TAC CTG CAC ATC CAG 3'

MN612 (SEQ.ID.NO:40) 5' CAT GGG GCT CAC GCA CAG CAG CTT GCT CAG CAG CTT CTC GAT GGT CTC GCG GTT CTT GCC GCA CTT GTA GCG CAC CAG CAG CAG CAC CAC CAT GCC CCA GCT GCA GGC CAG GCT CTG GAT GTG CAG GTA CAG GCA G 3'

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FIGURE 18, CTD. 2/3

MN613 (SEQ.ID.NO:41) 5' CTG CTG TGC GTG AGC CCC ATG TGC ATG ATG ATC GAG CCT CCC AAG CTT CGC AGC ACC GCC GCC GCC CTG TAC TGG TAC AAG ACC GGC ATC AGC AAC ATC AGC GAG GTG TAC GGC GAC ACC CCC GAG TGG ATC 3'

MN614 (SEQ.ID.NO:42) 5' GGC GAT CTC GCT GTC GTC CAC GAT GTC GTT GTC GTA GGC CCA CTG CAC CAT CTG GCT CAG CTC GAA GGT GCA GTC GTT GAA GCT GTG CTG CAG CAC GGT CTG GCG CTG GAT CCA CTC GGG GGT GTC GCC 3'

MN615 (SEQ.ID.NO:43): 5' GTG GAC GAC AGC GAG ATC GCC TAC AAG TAC GCC CAG CTG GCC GAC ACC AAC AGC AAC GCC AGC GCC TTC CTG AAG AGC AAC AGC CA GGC CAA GAT CGT GAA GGA CTG CGC CAC CAT GTG CCG CCA CTA C 3'

MN616 (SEQ.ID.NO:44) 5' GTA GCG CAG GAA CAT CAC GAT CTG CTT GCG GTC GCC GCC GTC GTC CAC GCG GTC GCA GCG GTA CTT GAT CCA CTG GCT CAT GCT CAT CTG CTT CTT CTC GGC GCG CTT GTA GTG GCG GCA CAT GGT GGC 3'

MN617 (SEQ.ID.NO:45) 5' CAG ATC GTG ATG TTC CTG CGC TAC CAG GGC GTG GAA TTC ATG AGC TTC CTG ACC GCC CTG AAG CGC TTC CTG CAG GGC ATC CCC AAG AAG AAC TGC ATC CTG CTG TAC GGC GCC GCC AAC ACC GAC AAG 3'

MN618 (SEQ.ID.NO:46) 5' GCC GAT CTT GGC GTC GGC CAG GGG CTG CAG CCA GAA GTG GCT CTT GCT GTT CAC GAA GCA GAT CAC GCT GCC CTG CAG GAA CTT CAT CAG GCT CAT GCC GAA CAG GCT CTT GTC GGT GTT GGC GGC GCCG 3'

MN619 (SEQ.ID.NO:47) 5' CTG GCC GAC GCC AAG ATC GGC ATG CTG GAC GAC GCC ACC GTG CCC TGC TGG AAC TAC ATC GAC GAC AAC CTG CGC AAC GCC CTG GAC GGC AAC CTG GTG AGC ATG GAC GTG AAG CAC CGC CCC CTG GTG 3'

MN620 (SEQ.ID.NO:48) 5' GAA CTC GTT GGG GAA GGT GAA CAC CAC CAG GCG GTT GTG CAG GTA GGG CCA GCG GCT GTC GGT GCC GGC GTT GAT GTT GCT GGT GAT CAG CAG GGG AGG GCA CTT CAG CTG CAC CAG GGG GCG GTG CTT CAC 3'

FIGURE 18, CTD 3/3

MN621 (SEQ.ID.NO:49) 5' GTG TTC ACC TTC CCC AAC GAG TTC CCC TTC GAC GAG AAC GGT AAC CCC GTG TAC GAG CTG AAC GAC AAG AAC TGG AAG AGC TTC TTC AGC CGC ACC TGG AGC CGC CTG AGC CTG CAC GAG GAC GAG 3'

MN622 (SEQ.ID.NO:50) 5' CAT GAG AGA TCT TTA CAG GGT GTT GGT GTT CTG GCC GCT CAC GCA CTT GAA GGT GGG CAG GCT GTC GCC GTC GTT CTC CTT GTC CTC GTC CTC GTG CAG GCT CAG 3'

MN623 (SEQ.ID.NO:51) 5' GCC TGA AGG CCA TCT GCA TCG AG 3'

MN624 (SEQ.ID.NO:52) 5' CTC GAT GCA GAT GGC CTT CAG GC 3'

MN625 (SEQ.ID.NO:53) 5' GAG CTG GTG CGC CCC TTC AAG 3'

MN626 (SEQ.ID.NO:54) 5' CTT GAA GGG GCG CAC CAG CTC 3'

MN627 (SEQ.ID.NO:55) 5' CTG CTG TGC GTG AGC CCC ATG 3'

MN628 (SEQ.ID.NO:56) 5' CAT GGG GCT CAC GCA CAG CAG 3'

MN629 (SEQ.ID.NO:57) 5' GCC ACC ATG TGC CGC CAC TAC 3'

MN630 (SEQ.ID.NO:58) 5' GTA GTG GCG GCA CAT GGT GGC 3'

MN631 (SEQ.ID.NO:59) 5' CTG GCC GAC GCC AAG ATC GGC 3'

MN632 (SEQ.ID.NO:60) 5' GCC GAT CTT GGC GTC GGC CAG 3'

MN633 (SEQ.ID.NO:61) 5' GTG TTC ACC TTC CCC AAC GAG TTC 3'

MN634 (SEQ.ID.NO:62) 5' GAA CTC GTT GGG GAA GGT GAA CAC 3'

MN635 (SEQ.ID.NO:63) 5' CAT GAG AGA TCT TTA CAG GGT GTT G 3'

MN636 (SEQ.ID.NO:64) 5' CAT CTC AGA TCT GCC ACC ATG GCC GAC CCC GCC GGC AC 3'

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FIGURE 19

Oligonucleotides used in the generation of synthetic HPV 16 E2

13856-307-2A (SEQ.ID.NO:65) 5' ATG GAG ACC CTG TGC CAG CGC CTG AAC GTG TGC CAG GAC AAG ATC CTG ACC CAC TAC GAG AAC GAC AGC ACC GAC CTG CGC GAC CAC ATC GAC TAC TGG 3'

13856-307-2C (SEQ.ID.NO:66) 5' CCA CCA GGT GGT GCC CAC CCT GGC CGT GAG CAA GAA CAA GGC CCT GCA GGC CGC CGA GCT GCA GCT GAC CCT GGA GAC GAT CTA CAA CAG CCA GTA CAG CAA CG 3'

13856-307-2E (SEQ.ID.NO:67) 5' CCG GCT GCA TCA AGA AGC ACG GCT ACA CCG TGG AGG TGC AGT TCG ACG GCG ACA TCT GCA ACA CCA TGC ACT ACA CCA ACT GGA CCC ACA TTT ACA TCT GTG AGG AGG 3'

13856-307-2G (SEQ.ID.NO:68) 5' CGT GCA CGA GGG GAT CCG CAC CTA CTT CGT GCA GTT CAA GGA CGA CGC CGA GAA GTA CAG CAA GAA CAA GGT GTG GGA GGT GCA CGC CGG AGG CCA GGT GAT CC 3'

13856-307-2I (SEQ.ID.NO:69) 5' GGC CAA CCA CAG CGC CGC CAC CCA CAC CAA GGC CGT GGC CCT GGG CAC CGA GGA GAC CCA GAC CAC AAT CCA GCG CCC TCG CAG CGA GCC CGA CAC CGG CAA CCC CTG CC 3'

13856-307-2K (SEQ.ID.NO:70) 5' GCC ACA AGG GCC GGA TCA ACT GCA ACA GCA ACA CCA CCC CTA TCG TGC ACC TGA AGG GCG ACG CCA ACA CCC TGA AGT GCC TGC GGT ACC GCT TCA AGA AGC ACT GC 3'

13856-307-2B (SEQ.ID.NO:71) 5' CCA GGG TGG GCA CCA CCT GGT GGT TGA TGT GCT TGA AGC CCA TCT CGC GGG CCT TGT AGT AGA TGG CGC AGG CCA GGC GCA TGT GCT TCC AGT AGT CGA TGT GGT CGC GCA GG 3'

13856-307-2D (SEQ.ID.NO:72) 5' GCC GTG CTT CTT GAT GCA GCC GGT AGG GGC GGT CAG GTA CAC CTC CAG GCT CAC GTC CTG CAG GGT CCA CTT CTC GTT GCT GTA CTG GCT GTT GTA GAT CG 3'

13856-307-2F (SEQ.ID.NO:73) 5' GGT GCG GAT CCC CTC GTG CAC GTA GTA CAG GCC GTA GTA GTC CAC CTG GCC CTC CAC CAC GGT CAC GCT GGC CTC CTC ACA GAT GTA AAT GTG GGT CC 3'

13856-307-2H (SEQ.ID.NO:74) 5' GGG TGG CGG CGC TGT GGT TGG CCA GGT GCT GGC GGA TCG TCT CGG GGC TGC TCA CCT CGT TGC TGC TGA ACA CGC TGG TGG GGC ACA GGA TCA CCT GGC CTC CGG CGT GC 3'

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FIGURE 19, CTD. 2/2

13856-307-2J (SEQ.ID.NO:75) 5' GCA GTT GAT CCG GCC CTT GTG GCT GCT GTT GAA GGC GGT CAG GAT AGG GGC GCT GTC GAC GCT GTC GCG GTG CAG CAG CTT GGT GGT GTG GCA GGG GTT GCC GGT GTC GGG 3'

13856-307-2L (SEQ.ID.NO:76) 5' CGT AGG TCA GGG TCA CGA TAG CGC TCT TGT GCT TCA CGT TGT GGC CGG TCC AGT GCC AGG TGC TGC TCA CGG CGG TGT ACA GCT TGC AGT GCT TCT TGA AGC GGT ACC GC 3'

13856-307-2M (SEQ.ID.NO:77) 5' TTT AGA TGC TCA TGA AGC CGG TGC TCA CGG TGA TGG TCT TGG GGA TCT TCA CCT GGC TCA GGA ACT GGT CGC GCT GCC ACT CGC TGT CGT AGG TCA GGG TCA CGA TAG CGC 3'

13856-307-2PA (SEQ.ID.NO:78) 5' CGA GCT GAT ATC GAA TTC AGA TCT GCC ACC ATG GAG ACC CTG TGC CAG CG 3'

13856-307-2PM (SEQ.ID.NO:79) 5' GGT TGC AGA TCT AGA CTC GAG TTT AGA TGC TCA TGA AGC CGG TGC 3'

13856-307-2PE (SEQ.ID.NO:80) 5' CCG GCT GCA TCA AGA AGC ACG 3'

13856-307-2PI (SEQ.ID.NO:81) 5' GGC CAA CCA CAG CGC CGC C 3'

13856-307-2PD (SEQ.ID.NO:82) 5' GCC GTG CTT CTT GAT GCA GCC 3'

13856-307-2PH (SEQ.ID.NO:83) 5' GGG TGG CGG CGC TGT GG 3'

13856-307-2PL (SEQ.ID.NO:84) 5' CGT AGG TCA GGG TCA CGA TAG C 3'

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FIGURE 20

Oligonucleotides used in the generation of synthetic HPV 16 E7.

13856-307-7A (SEQ.ID.NO:85) 5' GGC CGG AGA TCT GAT ATC GAA TTC GCC ACC ATG CAC GGC GAC ACC CCC ACC CTG CAC GAG TAC ATG CTG GAC CTG CAG CCC GAG ACC ACC GAC CTG TAC GGC TAC GGC C 3'

13856-307-7C (SEQ.ID.NO:86) 5' GCC GAG CCC GAC CGC GCC CAC TAC AAC ATC GTG ACC TTC TGC TGC AAG TGC GAC AGC ACC CTG CGC CTG TGC GTG CAG AGC ACC CAC GTC GAC ATC CGC ACC CTG G 3'

13856-307-7B (SEQ.ID.NO:87) 5' GGG CGC GGT CGG GCT CGG CCT GGC CGG CGG GGC CGT CGA TCT CGT CCT CTT CCT CGC TGC TGT CGT TCA GCT GGC CGT AGC CGT ACA GGT CGG TGG 3'

13856-307-7D (SEQ.ID.NO:88) 5' CCG CGG CAG ATC TAG ACT CGA GTT TAG GGC TTC TGG CTG CAG ATT GGG CAC ACG ATT CCC AGG GTG CCC ATC AGC AGG TCC TCC AGG GTG CGG ATG TCG ACG TGG G 3'

13856-307-7PA (SEQ.ID.NO:89) 5' GGC CGG AGA TCT GAT ATC GAA TTC G 3'

13856-307-7PD (SEQ.ID.NO:90) 5' CCG CGG CAG ATC TAG ACT CG 3'

FIGURE 21

Oligonucleotides Used for Construction of HPV6a E7 Gene

A. DNA Template Oligos

LS207 (105-mer) (SEQ.ID.NO:91) 5' GTC ACA GAT CTG ATA TCG AAT TCC ACC ATG CAC GGC CGC CAC GTG ACC CTG AAG GAC ATC GTG CTG GAC CTG CAG CCT CCC GAC CCC GTG GGC CTG CAC TGC TAC 3'

LS208 (105-mer) (SEQ.ID.NO:92) 5' CTG GAA GTG CTG CTT CAG GGG CTG GCT GTC CTG GCC GTC CAC CTC GTC CAC CTC GTC CTC GCT GCT GTC CAC CAG CTG CTC GTA GCA GTG CAG GCC CAC GGG GTC 3'

LS209 (107-mer) (SEQ.ID.NO:93) 5' CCA GCC CCT GAA GCA GCA CTT CCA GAT CGT GAC CTG CTG CTG CGG CTG CGA CAG CAA CGT GCG CCT GGT GGT GCA GTG CAC CGA GAC CGA CAT CCG CGA GGT GCA GC 3'

LS210 (102-mer) (SEQ.ID.NO:94) 5' CAG TCA GAT CTA GAG ATA TCT TTA GGT CTT GGG AGC GCA GAT GGG GCA CAC GAT GTT CAG GGT ACC CAG CAG GAG CTG CTG CAC CTC GCG GAT GTC GGT CTC 3'

B. PCR Amplification Primers

LS211 (24-mer) (SEQ.ID.NO:95) 5' GTC ACA GAT CTG ATA TCG AAT TCC 3'

LS212 (26-mer) (SEQ.ID.NO:96) 5' CAG TCA GAT CTA GAG ATA TCT TTA GG 3'

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FIGURE 22

Oligonucleotides Used for Construction of HPV18 E7 Gene

A. DNA Template Oligos

LS201 (109-mer) (SEQ.ID.NO:97) 5' GTC ACA GAT CTG ATA TCG AAT TCC ACC ATG CAC GGC CCC AAG GCC ACC CTG CAG GAC ATC GTG CTG CAC CTG GAG CCC CAG AAC GAG ATC CCC GTG GAC CTG CTG TGC C 3'

LS202 (111-mer) (SEQ.ID.NO:98) 5' GGG CTC GGC CCT GCG AGC GGG CAG GTG CTG GTG GTT CAC GCC GTC GAT CTC GTC GTT CTC CTC CTC GCT GTC GCT CAG CTG CTC GTG GCA CAG CAG GTC CAC GGG GAT CTC 3'

LS203 (108-mer) (SEQ.ID.NO:99) 5' GCC CGC TCG CAG GGC CGA GCC CCA GCG CCA CAC CAT GCT GTG CAT GTG CTG CAA GTG CGA GGC CCG CAT CGA GCT GGT GGT GGA GAG CAG CGC TGA CGA CCT GCG CGC 3'

LS204 (109-mer) (SEQ.ID.NO:100) 5' CAG TCA GAT CTA GAG ATA TCT TTA CTG CTG GCT GGC GCA CCA GGG GCA CAC GAA GCT CAG GGT GTT CAG GAA CAG CTG CTG GAA GGC GCG CAG GTC GTC AGC GCT GCT C 3'

B. PCR Amplification Primers

LS205 (26-mer) (SEQ.ID.NO:101) 5' GTC ACA GAT CTG ATA TCG AAT TCC AC 3'

LS206 (27-mer) (SEQ.ID.NO:102) 5' CAG TCA GAT CTA GAG ATA TCT TTA CTG 3'

FIGURE 23

Oligonucleotides used in the construction of HPV6 E2

6A 1-84 (90mer) (SEQ.ID.NO:103) 5' GAA TTC AGA TCT GAT ATC ACC ATG GAG GCC ATC GCC AAG CGC CTG GAC GCC TGC CAG GAG CAG CTG CTG GAG CTG TAC GAG GAG AAC AGC 3'

6B 65-157 (92mer) (SEQ.ID.NO:104) 5' CCT TGT ACA GCA GCA CGC TCT CGT GGC GCA TGC ACT TCC AGT GCA GCA CGT GCT TGT GCA GGT CGG TGC TGT TCT CCT CGT ACA GCT CCA GC 3'

6C 132-227 (96mer) (SEQ.ID.NO:105) 5' CCA CGA GAG CGT GCT GCT GTA CAA GGC CAA GCA GAT GGG CCT GAG CCA CAT CGG CAT GCA GGT GGT GCC TCC TCT GAA GGT GAG CGA GGC CAA GGG 3'

6D 202-304 (103mer) (SEQ.ID.NO:106) 5' GCA GGG TCC AGG GCT CCA TGC TGT ACT CGG TGC GCA GCA GGC TCT CGA GGT GCA TCT GCA TCT CGA TGG CGT TGT GGC CCT TGG CCT CGC TCA CCT TCA GAG G 3'

6E 276-373 (98mer) (SEQ.ID.NO:107) 5' CGA GTA CAG CAT GGA GCC CTG GAC CCT GCA GGA GAC CAG CTA CGA GAT GTG GCA GAC CCC TCC CAA GCG CTG CTT CAA GAA GCG CGG CAA GAC CGT GG 3'

6F 347-448 (102mer) (SEQ.ID.NO:108) 5' CGT TGT CCT GCA CGT ACA CGT CGG TCC ACA CCA CGT AGT CCA TGG TGT TGT TGG CGC AGC CGT CGA ACT TCA CCT CCA CGG TCT TGC CGC GCT TCT TGA AGC 3'

6G 425-526 (102mer) (SEQ.ID.NO:109) 5' CCG ACG TGT ACG TGC AGG ACA ACG ACA CCT GGG TGA AGG TGC ACA GCA TGG TGG ACG CCA AGG GCA TCT ACT ACA CCT GTG GCC AGT TCA AGA CCT ACT ACG 3'

6H 495-586 (92mer) (SEQ.ID.NO:110) 5' GCT GCC GTA GCA CAC CTC CCA GTG CTT GGT GCT GCC GTA CTT CTC GGC CTC CTT CAC GAA GTT CAC GTA GTA GGT CTT GAA CTG GCC ACA GG 3'

6I 500-591 (94mer) (SEQ.ID.NO:111) 5' GCA CTG GGA GGT GTG CTA CGG CAG CAC CGT GAT CTG CAG CCC CGC TAG CGT GAG CAG CAC CAC CCA GGA GGT GAG CAT CCC CGA GAG CAC CAC C 3'

6J 636-732 (97mer) (SEQ.ID.NO:112) 5' GCG AGG AGG GGT CTG CAC GGC GTC CTC CTT GGT GCT GCT GCT CAC CAG GGT GCT GGT CTG GGC GGG AGT GTA GGT GGT GCT CTC GGG GAT GCT CAC C 3'

FIGURE 23, CTD. 2/2

6K 708-804 (97mer) (SEQ.ID.NO:113) 5' GGA CGC CGT GCA GAC CCC TCC TCG CAA GCG CGC CCG CGG CGT GCA GCA GAG CCC CTG CAA CGC CCT GTG CGT GGC CCA CAT CGG CCC CGT GGA CAG C 3'

6L 780-873 (94mer) (SEQ.ID.NO:114) 5' GGC GCT GCT GTT GCT GTT GTT GCG GCG CTG GTG CTG GTC GTG GTT GTT GGT GAT CAG GTT GTG GTT GCC GCT GTC CAC GGG GCC GAT GTG GGC C 3'

6M 849-943 (95mer) (SEQ.ID.NO:115) 5' CCG CAA CAA CAG CAA CAG CAG CGC CAC TCC CAT CGT GCA GTT CCA GGG CGA GAG CAA CTG CCT GAA GTG CTT CCG CTA CCG CCT GAA CGA TCG CC 3'

6N 917-1012 (96mer) (SEQ.ID.NO:116) 5' CGT GCT TGT GGG GAG CCT TGC TGC TGG CCC AGT GCC AGG TGC TGC TGA TCA GGT CGA ACA GGT GGC GGT GGC GAT CGT TCA GGC GGT AGC GGA AGC 3'

6O 989-1083 (95mer) (SEQ.ID.NO:117) 5' GCA GCA AGG CTC CCC ACA AGC ACG CCA TCG TGA CCG TGA CCT ACG ACA GCG AGG AGC AGC GCC AGC AGT TCC TGG ACG TGG TGA AGA TCC CTC CC 3'

6P 1059-1154 (96mer) (SEQ.ID.NO:118) 5' CTC GAG AGA TCT CCC GGG TCT AGA GCT TAC AGC AGG TGC AGG CTC ATG AAG CCC AGC TTG TGG CTG ATG GTG GGA GGG ATC TTC ACC ACG TCC AGG 3'

6PA 25mer (SEQ.ID.NO:119) 5' GAA TTC AGA TCT GAT ATC ACC ATG G 3'

6PD 21mer (SEQ.ID.NO:120) 5' GCA GGG TCC AGG GCT CCA TGC 3'

6PE 25mer (SEQ.ID.NO:121) 5' CGA GTA CAG CAT GGA GCC CTG GAC C 3'

6PH 25mer (SEQ.ID.NO:122) 5' GCT GCC GTA GCA CAC CTC CCA GTG C 3'

6PI 21mer (SEQ.ID.NO:123) 5' GCA CTG GGA GGT GTG CTA CGG 3'

6PL 23mer (SEQ.ID.NO:124) 5' GGC GCT GCT GTT GCT GTT GTT GC 3'

6PM 22mer (SEQ.ID.NO:125) 5' CCG CAA CAA CAG CAA CAG CAG C 3'

6PP 26mer (SEQ.ID.NO:126) 5' CTC GAG AGA TCT CCC GGG TCT AGA GC 3'

FIGURE 24

Oligonucleotides used to construct HPV18 E2

18A 1-97 (97mer) (SEQ.ID.NO:127) 5' GAA TTC AGA TCT GAT ATC ACC ATG CAG ACT CCC AAG GAG ACC CTG AGC GAG CGC CTG AGC GCC CTG CAG GA CAA GAT CAT CGA CCA CTA CGA GAA CG 3'

18B 69-166 (98mer) (SEQ.ID.NO:128) 5' CGA AGA AGA TGG CGT TCT CCC AGC GGA TCA GCT GCC AGT ACT GGA TCT GGC TGT CGA TGT CCT TGC TGT CGT TCT CGT AGT GGT CGA TGA TCT TGT CC 3'

18C 141-234 (94mer) (SEQ.ID.NO:129) 5' CCG CTG GGA GAA CGC CAT CTT CTT CGC CGC TCG CGA GCA CGG GAT CCA GAC CCT GAA CCA CCA GGT GGT GCC CGC CTA CAA CAT CAG CAA GAG C 3'

18D 211-304 (94mer) (SEQ.ID.NO:130) 5' CCT CGG TCT TGT AGG CGC TCT GGG CCA GGC CCT GCA GGG CCA TCT GCA GCT CGA TGG CCT TGT GGG CCT TGC TCT TGC TGA TGT TGT AGG CGG G 3'

18E 281-371 (91mer) (SEQ.ID.NO:131) 5' CCC AGA GCG CCT ACA AGA CCG AGG ACT GGA CCC TGC AGG ACA CCT GCG AGG AGC TGT GGA ACA CCG AGC CCA CCC ACT GCT TCA AGA AGG G 3'

18F 348-441 (94mer) (SEQ.ID.NO:132) 5' GCT GTC CCA GGC CAC GTA GTT CAT GCA GTT GTC CTT GTT GCC GTC GAA GTA CAC CTG CAC GGT CTG GCC TCC CTT CTT GAA GCA GTG GGT GGG C 3'

18G 416-505 (90mer) (SEQ.ID.NO:133) 5' GCA TGA ACT ACG TGG CCT GGG ACA GCG TGT ACT ACA TGA CCG ACG CCG GCA CCT GGG ACA AGA CCG CCA CCT GCG TGA GCC ACC GCG GCC 3'

18H 481-572 (92mer) (SEQ.ID.NO:134) 5' CCG TAC TTC TCG CAC TCG CTC TTG AAC TCG ATG TAG AAG GTG TTG TAG CCC TCC TTC ACG TAG TAC AGG CCG CGG TGG CTC ACG CAG GTG GC 3'

18I 543-636 (94mer) (SEQ.ID.NO:135) 5' CGA GTT CAA GAG CGA GTG CGA GAA GTA CGG CAA CAC CGG CAC CTG GGA GGT GCA CTT CGG CAA CAA CGT GAT CGA CTG CAA CGA CAG CAT GTG C 3'

18J 609-708 (100mer) (SEQ.ID.NO:136) 5' GCT GTA GGG GCT GGG AGT GTG CTG CAG CTG CTT CAC CAG CTG GGT GGC GCT CAC GGT GTC GTC GCT GGT GCT GCA CAT GCT GTC GTT GCA GTC GAT CAC G 3'

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FIGURE 24, CTD. 2/2

18K 687-779 (93mer) (SEQ.ID.NO:137) 5' GCA CAC TCC CAG CCC CTA CAG CAG CAC CGT GAG CGT GGG CAC CGC CAA GAC CTA CGG CCA GAC CAG CGC CGC CAC TCG CCC TGG CCA CTG CGG 3'

18L 758-853 (96mer) (SEQ.ID.NO:138) 5' GCT TGT TGT TGC CGG TGG CGG TGG CGG CGC CCA GCA GAG GGT TCA CGG GCC CGC AGT GCT GCT TCT CGG CCA GGC CGC AGT GGC CAG GGC GAG TGG 3'

18M 829-925 (97mer) (SEQ.ID.NO:139) 5' GCC ACC GCC ACC GGC AAC AAC AAG CGC CGC AAG CTG TGC AGC GGC AAC ACC ACT CCC ATC ATC CAC CTG AAG GGC GAC CGC AAC AGC CTG AAG TGC C 3'

18N 900-996 (97mer) (SEQ.ID.NO:140) 5' GGC GCC GGT CCA GTG CCA GGT GCT GCT GAT GTC GCG GTA GTG GTC GCT GTG CTT GCG CAG GCG GTA CCG CAG GCA CTT CAG GCT GTT GCG GTC GCC C 3'

18O 974-1072 (99mer) (SEQ.ID.NO:141) 5' GCA CCT GGC ACT GGA CCG GCG CCG GGA ACG AGA AGA CCG GCA TCC TGA CCG TGA CCT ACC ACA GCG AGA CCC AGC GCA CCA AGT TCC TGA ACA CCG TGG 3'

18P 1048-1145 (98mer) (SEQ.ID.NO:142) 5' CTC GAG AGA TCT CCC GGG TCT AGA GCT TAC ATG GTC ATG TAG CCC ACC AGG ATC TGC ACG CTG TCG GGG ATG GCC ACG GTG TTC AGG AAC TTG GTG CG 3'

18PA 25mer (SEQ.ID.NO:143) 5' GAA TTC AGA TCT GAT ATC ACC ATG C 3'

18PD 23mer (SEQ.ID.NO:144) 5' CCT CGG TCT TGT AGG CGC TCT GG 3'

18PE 21mer (SEQ.ID.NO:145) 5' CCC AGA GCG CCT ACA AGA CCG 3'

18PH 21mer (SEQ.ID.NO:146) 5' CCG TAC TTC TCG CAC TCG CTC 3'

18PI 20mer (SEQ.ID.NO:147) 5' CGA GTT CAA GAG CGA GTG CG 3'

18PL 21mer (SEQ.ID.NO:148) 5' GCT TGT TGT TGC CGG TGG CGG 3'

18PM 25mer (SEQ.ID.NO:149) 5' GCC ACC GCC ACC GGC AAC AAC AAG C 3'

18PP 26mer (SEQ.ID.NO:150) 5' CTC GAG AGA TCT CCC GGG TCT AGA GC 3'

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FIGURE 25

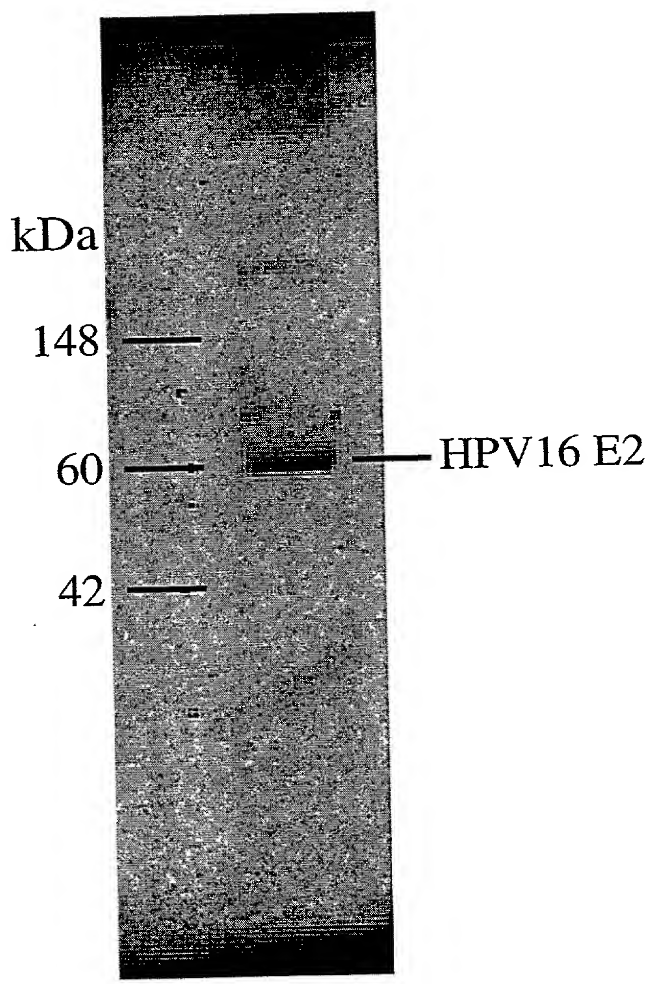
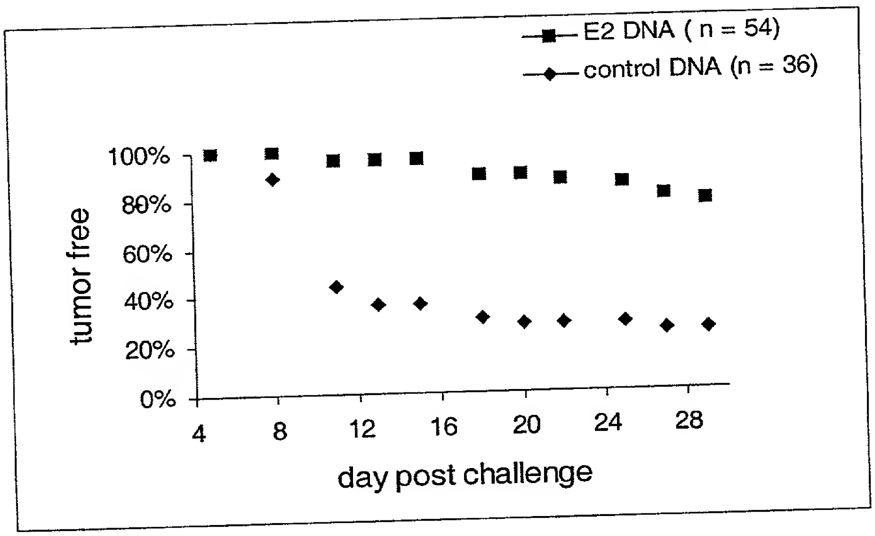


FIGURE 26


E2 DNA (n = 54)
control DNA (n = 36)
tumor free
100%
80%
60%
40%
20%
0%
4
8
12
16
20
24
28
day post challenge

DECLARATION AND POWER OF ATTORNEY FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

[X] Declaration Submitted with Initial Filing **OR** [ ] Declaration Submitted after Initial Filing (surcharge (37 CFR 1 16 (e)) required)

Attorney Docket Number	20413Y
First Named Inventor	M. NEEPER ET AL.
COMPLETE IF KNOWN	
Application Number	
Filing Date	August 21, 2000
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

SYNTHETIC HUMAN PAPILLOMAVIRUS GENES

(Title of the Invention)

the specification of which

[X] is attached hereto

OR

[ ] was filed on (MM/DD/YYYY) [ ] as United States Application Number or PCT International Application Number [ ] and was amended on (MM/DD/YYYY) [ ] (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Attorney Docket Number	Priority Claimed? YES	NO
				[ ]	[ ]
				[ ]	[ ]
				[ ]	[ ]
				[ ]	[ ]

[ ] Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

I hereby claim the benefit under 35 U.S C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	Attorney Docket Number
60/150,728	08/25/1999	20413PV
60/210,143	06/07/2000	20413PV2

Computer generated form " Declaration Pwr of Atty" (Application Filing Folder), Merck & Co., Inc. 05/23/2000

Please type a plus sign (+) inside this box → [+]

Approved for use through 9/30/2000. OMB 651-0032

SUBSTITUTE for PTO/SB/01 (12-97), DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

DECLARATION AND POWER OF ATTORNEY for Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information known to me to be material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Application Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint, respectively and individually, as my attorneys or agents with full power of substitution and revocation, the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*

OR

☒ Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number
JOANNE M. GIESSER	32,838	JACK L. TRIBBLE	32,636

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Name	JOANNE M. GIESSER				
Address	Merck & Co., Inc. - Patent Department				
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
MICHAEL P	NEEPER

Inventor's Signature	X [signature]					**Date**	X 18 July 2000
Residence: City	COLLEGEVILLE	**State**	PA	**Country**	US	**Citizenship**	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway			**State**	NJ	**ZIP**	07065-0907

☒ Additional inventors are being named on the ____ supplemental Additional Inventors(s) sheet(s) PTO/SB/02A attached hereto.

DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S)
Supplemental Sheet

Name of Additional Joint Inventor, if any:				☐ A petition has been filed for this unsigned inventor			
Given Name (first and middle [if any])				Family Name or Surname			
WILLIAM L.				McCLEMENTS			
Inventor's Signature	X [signature]				**Date**	X 18 July 2000	
Residence: City	DOYLESTOWN	**State**	PA	**Country** US		**Citizenship**	US
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Name of Additional Joint Inventor, if any:				☐ A petition has been filed for this unsigned inventor			
Given Name (first and middle [if any])				Family Name or Surname			
KATHRIN U.				JANSEN			
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Residence: City	DOYLESTOWN	**State**	PA	**Country** US		**Citizenship**	GERMAN
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
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Name of Additional Joint Inventor, if any:				☐ A petition has been filed for this unsigned inventor			
Given Name (first and middle [if any])				Family Name or Surname			
LOREN D.				SCHULTZ			
Inventor's Signature	X [signature]				**Date**	X 18 July 2000	
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City	Rahway	**State**	NJ	**ZIP**	07065-0907		
Name of Additional Joint Inventor, if any:				☐ A petition has been filed for this unsigned inventor			
Given Name (first and middle [if any])				Family Name or Surname			
LING				CHEN			
Inventor's Signature	X [signature]				**Date**	X July 26, 2000	
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SUBSTITUTE for PTO/SB/02A (3-97), Declaration (Additional Inventors)

DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S)
Supplemental Sheet

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])				Family Name or Surname			
XIN-MIN				WANG			
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Post Office Address	Merck & Co., Inc., P.O. Box 2000						
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Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])				Family Name or Surname			
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Residence: City		**State**		**Country**		**Citizenship**	
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	**State**	NJ	**ZIP**	07065-0907		

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Given Name (first and middle [if any])				Family Name or Surname			
Inventor's Signature					**Date**		
Residence: City		**State**		**Country**		**Citizenship**	
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
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Given Name (first and middle [if any])				Family Name or Surname			
Inventor's Signature					**Date**		
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